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Nitrous acid deamination reactions of benzyl amino-4,6-0-benzylidene-D-hexopyranosides

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NITROUS ACID DEAMINATION REACTIONS OF BENZYL AMINO-4,6-O-BENZYLIDENE-D-HEXOPYRANOSIDES

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by

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CHAPTER I

INTRODUCTION

GENERAL

This study concerns an investigation of nitrous acid deamination of various amino sugar derivatives. Deamination reagents have also been used for the diazotization of aromatic compounds [33-37, 46]. Nitrous acid deamination was used in the deamination of amino acids [12], pinacolic amino alcohols [10, 50] and amino sugars [18, 62, 64].

Two major reaction pathways for deamination by nitrous acid have been proposed; in the first, diazonium ions and in the second, carbonium ions (from diazonium ions via loss of nitrogen), are important in determining the product composition of deamination reactions. Work on the mechanisms of the diazotization or deamination reaction is scarce. In some cases, the attacking entity is NO^+ and in others, it is apparently NOCl, NOBr, $NO_2H_2^+$, N_2O_3, etc., each of which can be viewed as a carrier of NO^+.

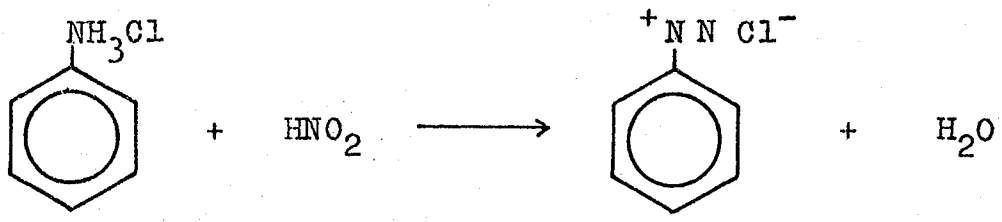
Resonance of the diazonium group with an aromatic ring stabilizes the ion and makes it relatively stable to nitrogen loss. Such aromatic diazonium ions would be more stable as compared to aliphatic diazonium ions.

AROMATIC DIAZONIUM IONS

A kinetic study of the formation of the benzenediazonium ion was done by Schmid and Muhr (1937) [35], and Ingold et al. (1958) [33-37]. The mechanism suggested by the authors is summarized in Figure I.

$$2HNO_2 \underset{}{\overset{K_e}{\rightleftharpoons}} H_2O + N_2O_3 \quad \text{(fast, equilibrium)}$$

$$\frac{[N_2O_3][H_2O]}{[HNO_2]^2} = K_e \quad , \quad [N_2O_3] = \frac{K_e}{[H_2O]} [HNO_2]^2$$



Aniline hydrochloride — Diazonium ion

$$\text{rate} = k [N_2O_3][PhNH_2] = k' [HNO_2]^2 [PhNH_2]$$

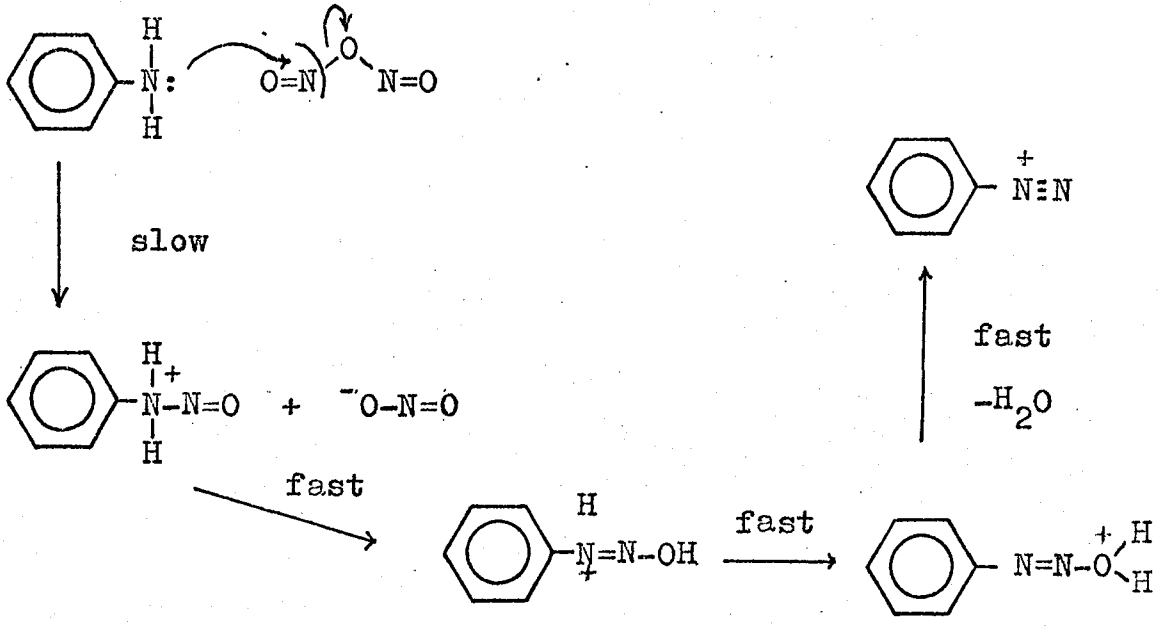


FIGURE I. Mechanism of the formation of benzenediazonium ion

Hughes et al. (1958) [33-37] found that the overall kinetic order for aniline diazotization varied from 3 to 3.6. When the aniline concentration was increased, its order fell from 1 to 0. However, the rate was always proportional to HNO_2^2, which provided evidence for N_2O_3 as the attacking species.

Attempts to control the rate of diazotization of aniline by acid catalysis were not very successful. Also, the reaction was found to be independent of anion concentrations [33]. In weakly acidic aqueous media, the diazotizing species were found to be nitrous acidium ion $H_2NO_2^+$, dinitrogen trioxide N_2O_3, nitrosyl chloride NOCl, and other nitrosyl halides. The nitrosonium ion NO^+, dinitrogen tetroxide N_2O_4, and nitrous acid HNO_2 were found to be ineffective in nitrosation. The possible attacking species in nitrous acid deamination are summarized in Figure II.

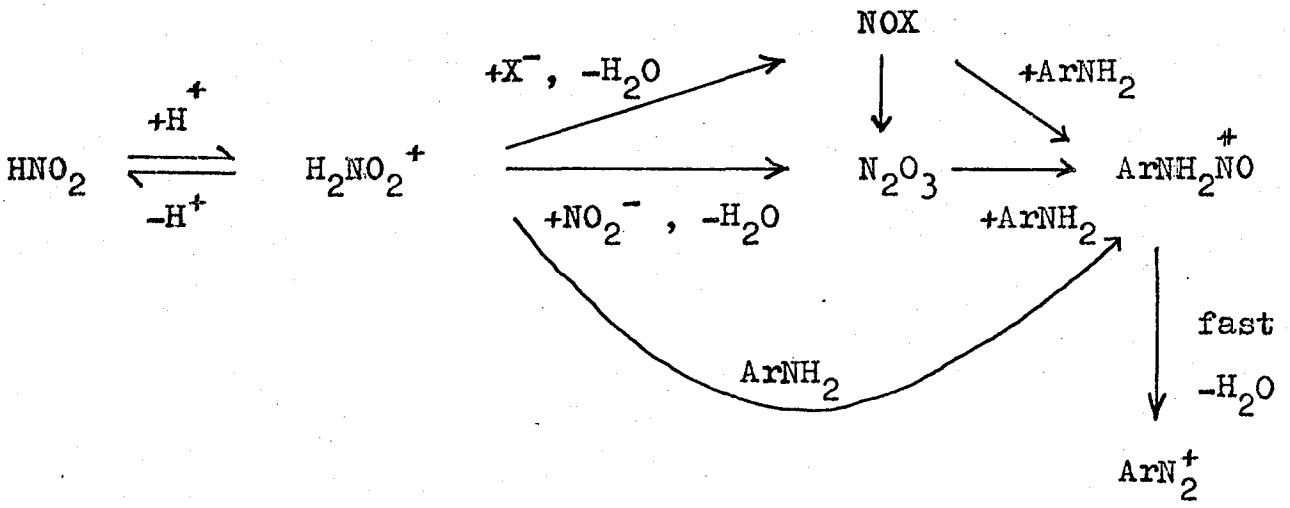


FIGURE II. Mechanism of aqueous diazotization of aniline

Aromatic diazonium ions can easily be coupled to another ring compound to form a diazo compound. However, such coupling is not found in most of the aliphatic diazonium ions which are less stable than the aromatic diazonium ions.

DEAMINATION OF ALIPHATIC AMINES

Stabilities of alkyl diazonium ions were first determined when PMR and fluorine NMR spectra could be obtained for more stable alkyl diazonium ions with electron withdrawing substituents [41, 54]. Stabilization could be achieved by fluorine substitution [54] as shown in Figure III.

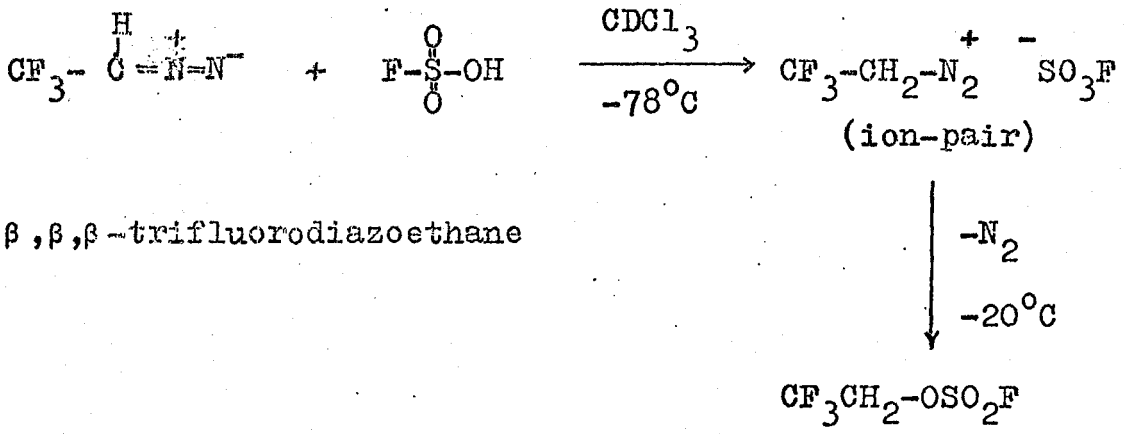


FIGURE III. Fluorine stabilization of an aliphatic diazonium ion

Also, an adjacent carbonyl group was found to stabilize an alkyl diazonium ion against nitrogen loss [54]. This is illustrated in Figure IV.

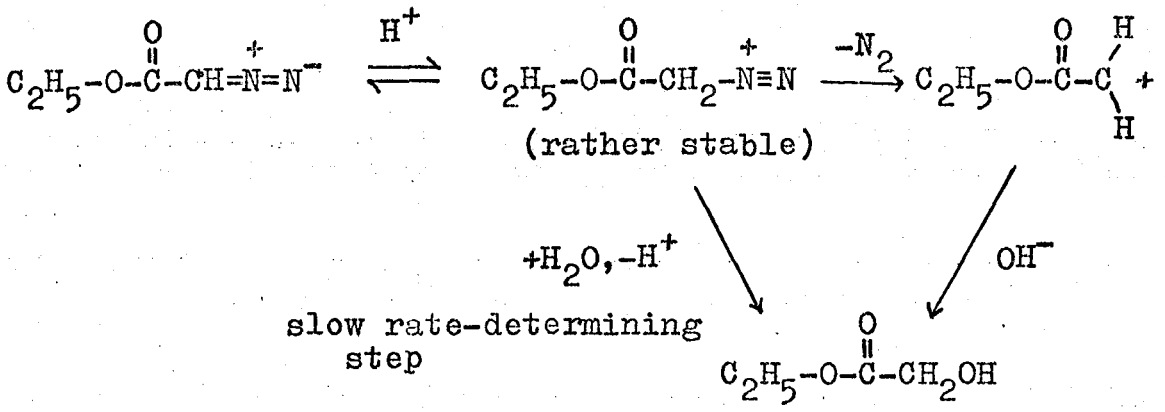


FIGURE IV. Carbonyl stabilization of an aliphatic diazonium ion

For the deamination reaction of primary aliphatic amines, Ingold suggested the diazonium ion as the major intermediate. The reaction was shown to depend kinetically on a process ending in N-nitrosation giving a nitroso-ammonium ion. The process leading to nitrosation would consist of several steps and take different courses depending on what kind of nitrosating agent was used. Various kinetic forms were worked out [33-37].

In a buffer system, maintained at pH 5, the overall kinetic order of nitrous acid fell from 2 to 1.8 [35a]. When operating in an acidic medium with a low concentration of nitrous acid, the reaction had first order in hydrogen ion, amine and nitrous acid.[35].

The deamination process was thought to go through a SN_2-like mechanism. However, Streitwieser (1957) [66] proposed a carbonium ion as the major intermediate in the deamination of primary aliphatic amines. According to Streitwieser, diazonium ions were rather unstable because of the stability of the leaving group, the nitrogen molecule. Consequently, the decomposition of an alkyldiazonium ion into nitrogen and carbonium ion required only a low activation energyof the order of 3 - 5 Kcal/mole.[66]. Also, the reaction would sometimes be exothermic. The transition state for the decomposition of an alkyldiazonium ion resembles more closely the reactant than the product. Thus, the scale of energy differences for competing reaction mechanism pathways would be expected to be compressed. A number of different routes would compete more or less successfully depending upon experimental conditions. Thus, the nitrous acid deamination of primary aliphatic amines was known to yield products resulting from solvolysis, elimination, substitution, and rearrangement, as indicated in Figure V.

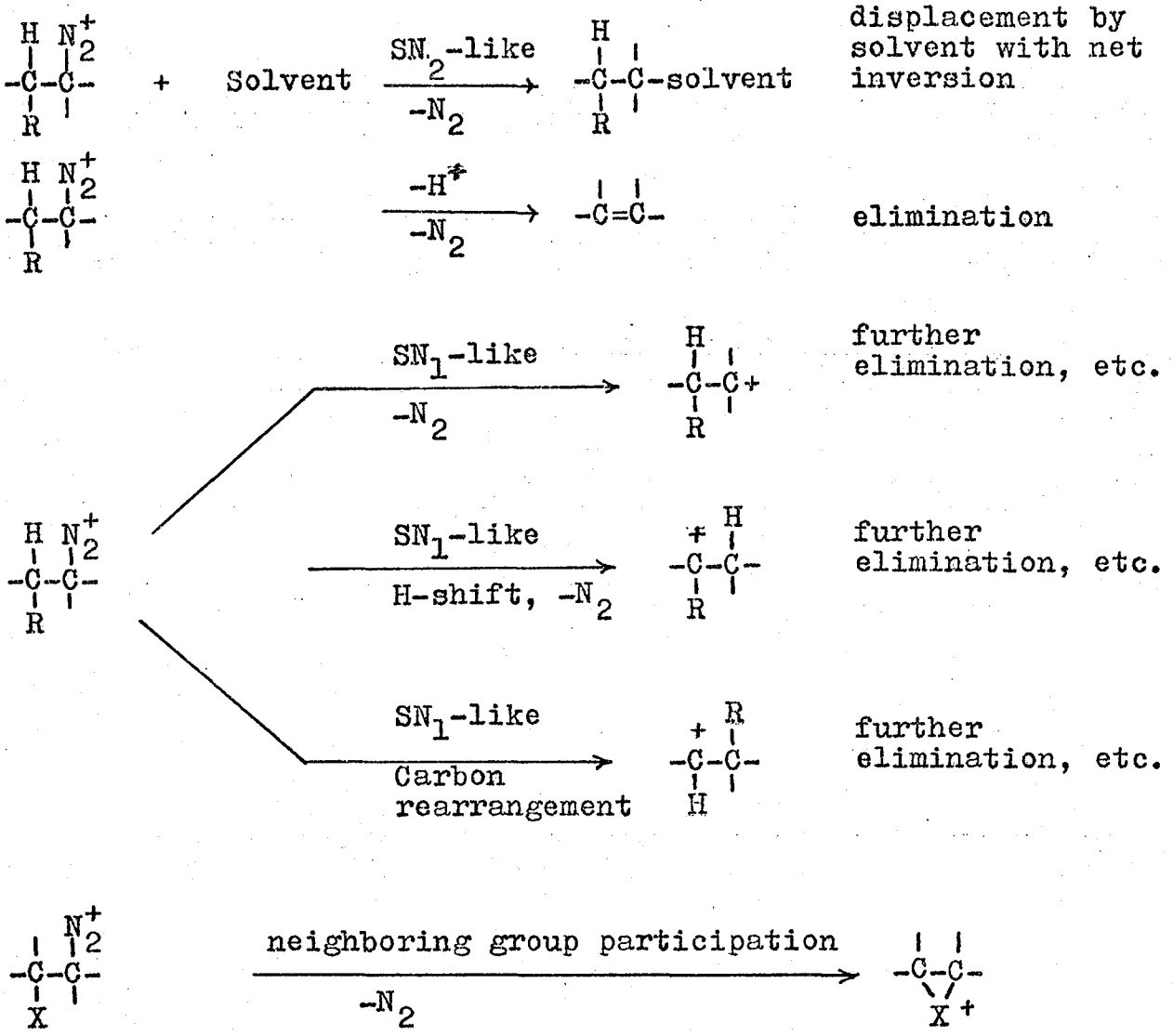


FIGURE V. General scheme for the fate of alkyldiazonium ions

In primary amines, those with a branch on carbon carrying an amino group would yield from direct displacement less inverted products than those with no branches. This is due to steric hindrance of adjacent groups. Thus, a more substituted optically active amine, such as sec-butylamine, and α-phenylethylamine, gives a more racemized product and the reactivity in direct displacement reactions decreases along the series: n-butyl>sec-butyl>α-phenylethyl [67].

In non-aqueous deamination, ion-pairs were thought to be the major intermediates. According to Olah and Schleyer [59, 60], such ionic pairs existed for the benzenediazonium hexafluorophosphate salt $PhN_2^+ \ PF_6^-$. In this context, Fairbrother and Wright's determination of the extent of association of a trityl halide with a Lewis acid [22] is also of interest.

$$Ph_3C\text{-}Br + SnBr \underset{}{\overset{K_e}{\rightleftharpoons}} Ph_3C^+ \ SnBr_5^- \longrightarrow Ph_3C^+ + SnBr_5^-$$

ionic pair

It is possible that carbonium ion pairing is responsible for a double inversion, and consequently net retention takes place. Brewster et al. [5] postulated

that solvated ion pairs play a role in partial net retention of configuration in the reaction of α-phenylethylamine with nitrous acid in aqueous acetic acid.

Solvolysis always happens in the presence of polar solvents where hydrogen-bonding is effective. According to Moss and Reger [53], the ratio of inverted and retention products depended on the critical micelle concentration (c.m.c.) of the reactants. When the concentration of the reactant was below the c.m.c., the freely solvated ions would react to give a more net inverted compound. When the concentration of the amine was higher than the c.m.c., micelle aggregates would give a higher net retention of configration. Friedman [25] postulated that generally more protic rearrangement would occur in polar reaction media because solvation increases stability and longevity of carbonium ions and decreases nucleophilicities of the counter-ion.

The elimination product composition obtained from sec-butylamine in aqueous acid [65], was considerably different from the composition of products obtained from acetolysis. The elimination requires the presence of an α-hydrogen anti-parallel and coplanar to the diazonium ion.

In the distribution of possibly formed conformers, the more preferred and more stable form would determine the dominant product in the final olefin mixture as was observed in the case of sec-butylamine [65]. When more conformers could be formed, more elimination products would be observed.

Hydrogen migration could also happen when the leaving group is anti-coplanar to an α-hydrogen. Migration of such an α-hydrogen could actually be a concerted reaction step leading to a hydrogen-bridged intermediate [4, 10], which can subsequently eliminate a proton to complete rearrangement.

The carbonium ion suggested by Streitwieser [66] is commonly postulated for SN_1-type reactions. Cram and McCarty [16] suggested the formation of "hot" carbonium ions from the decomposition of diazonium ions. These high energy intermediates would react quickly to give various displacement and elimination products. Such proposal cannot explain why certain deamination reactions are so stereoselective. Halmann and Roberts [31] presented evidence for "nonclassical" carbonium ion intermediates in the nitrous acid deamination of aliphatic amines.

The model of a "nonclassical carbonium ion" intermediate is most successful in the explanation of the stereochemical requirements for the breaking of one C-C bond and the formation of another. The migrating group and the leaving group must be oriented anticoplanar during the early stages of bond breaking. As the bond is broken, the positive charge of the incipient carbonium ion is shared by the migrating group. Examples of carbon migration are known from the Demjanov ring expansion [26], from the formation of sec-butyl alcohol from iso-butylamine [57], and from semipinacolic deaminations [48].

Classical valence structures can be written, if the migrating group is aromatic [13, 14, 15]. Neighboring group reaction can occur with anticoplanar heteroatoms [27].

It is always debatable whether there is a "non-classical" bridged [31, 58], or "classical" (open) [66], or "hot" carbonium ion [16], and whether there are two or perhaps three such intermediates interceding between reactant and product. Investigations should be done in individual cases to confirm the correct intermediates present in the reaction.

An extensive review of major types of carbonium ions was done by Olah and Schleyer [57] and Winstein [72]. Benzylic or allylic carbonium ions as well as "electron-sufficient" [6] bridged carbonium ions involve π electron delocalization. On the other hand, "electron-deficient" nonclassical carbonium cations involve, at least in part, overlap with the orbitals of carbon-carbon σ-bonds. Examples of such nonclassical intermediates can be seen in the following deamination reactions.

The deamination of 1-^{14}C -n-propylammonium perchlorate by nitrous acid gave 1-propanol in which 8% of the label was rearranged to C2 and C3 [31]. Although less than 8% rearrangement was observed, the results were consistent with the intervention of a protonated cyclopropane intermediate. Hydride shift studies for the deamination of C-tritiated amines, by NMR spectroscopy of the products and reactants, were done by several groups of workers [8]. The three carbon atoms in the proposed intermediates were thought to approach a state almost chemically equivalent to give a "nonclassical" carbonium ion, as shown in Figure VI.

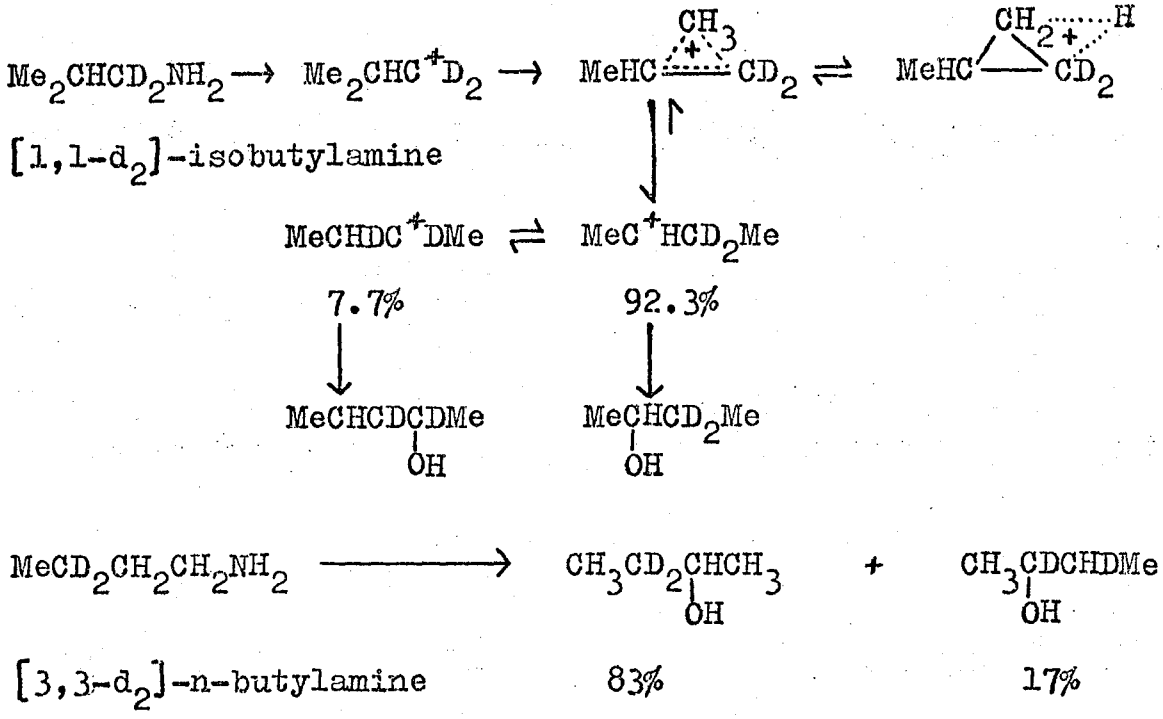


FIGURE VI. Deamination of primary aliphatic amines

SEMIPINACOLIC DEAMINATION

The deamination of β-aminohydrins gave products equivalent to those resulting from pinacol rearrangements. It is very likely that mechanisms for both are very similar, involving carbon function rearrangement.

In the semipinacolic deamination[28a], a compound such as (I) in Figure VII would form the corresponding

ketone (II). The product formed depended on the size of R, which would determine the most stable conformation.

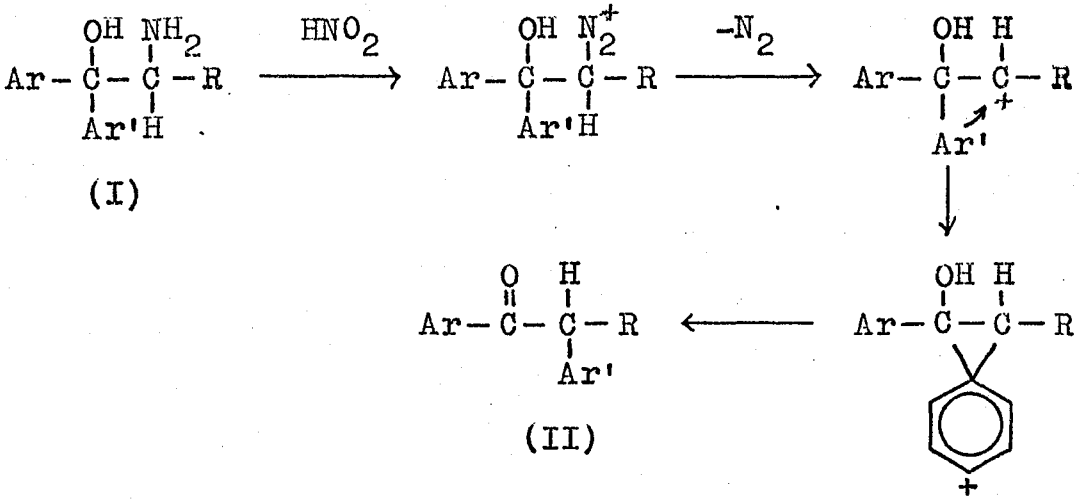


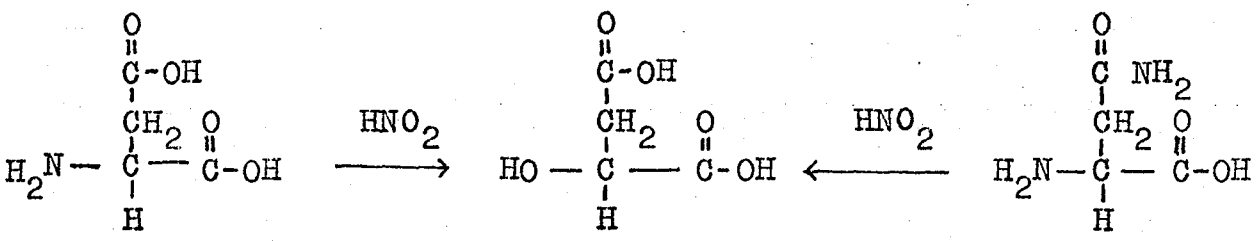
FIGURE VII. Semipinacolic rearrangement of β-aminohydrin

The deamination of such an aromatic β-aminohydrin is a typical example of neighboring group participation by an aromatic ring. Neighboring participation is also observed for adjacent oxygen functions.

DEAMINATION OF AMINO ACIDS

The deamination of α-amino acids with nitrous acid gave products with net retention of configuration [63].

Presumably, α-lactones were formed with inversion, which was followed by another inversion in the reaction of these α-lactones with solvent. The result was net retention of configuration for resulting hydroxy acid [5]. As shown in Figure VIII, asparagine and aspartic acid were converted to malic acid.



Aspartic acid Malic acid Asparagine

FIGURE VIII. Deamination of α-amino acid

Similarly, retention of configuration in other deamination products is found to be caused by double inversion, with the solvent or the neighboring groups participating. Such mechanisms would be particularly important in cases of cyclic systems having amino group and hydroxyl group on adjacent carbon atoms.

DEAMINATION OF CYCLIC AMINES

Cis-cyclohexanol with almost complete retention of configuration was obtained from cis-cyclohexylamine-2-d with aqueous nitrous acid (Streitwieser, 1959) [12]. Such retention could have resulted from front side attack of a water molecule, attached with a hydrogen-bond to the diazotic acid-^{18}O [52, 54] as shown in Figure IX.

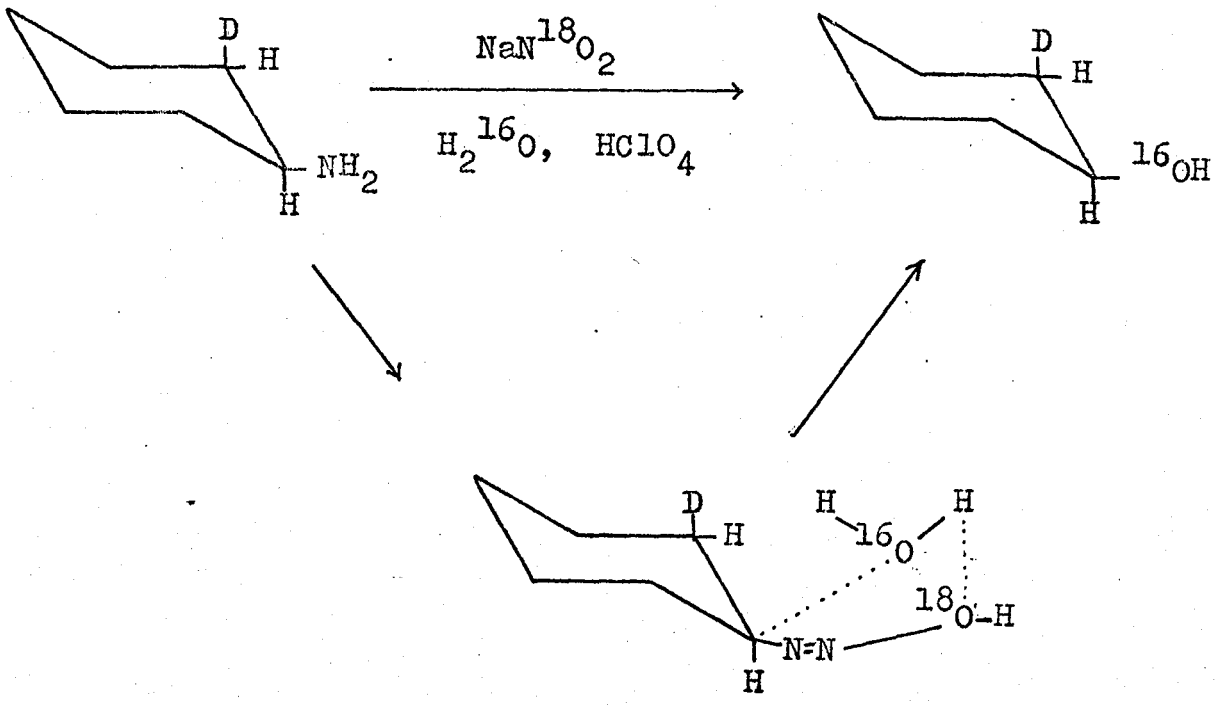
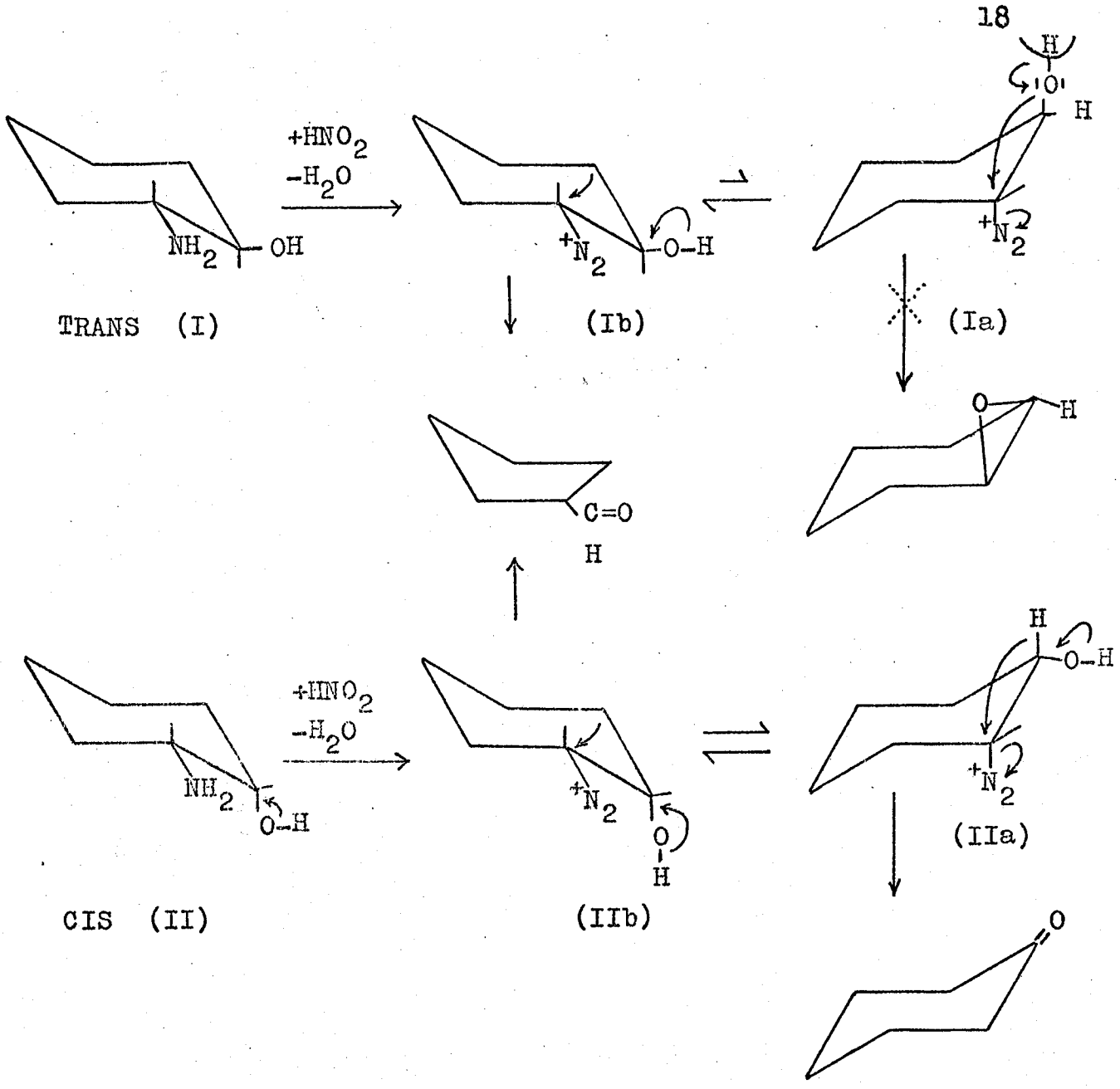


FIGURE IX. Water participation in deamination of cyclohexylamine-2-d

DEAMINATION IN CYCLIC SEMIPINACOLIC SYSTEMS

Neighboring group participation was found for semipinacolic rearrangements of cyclic compounds. 2-aminocyclohexanols, on treatment with nitrous acid, gave a ketone and a ring-contracted aldehyde [50]. An anti-coplanar relationship between the leaving diazonium ion and the migrating bond was postulated as a requirement for such rearrangement.

The major product arose from reactions between the cationic center and bonds at the carbon with the hydroxyl group. In Figure X, the conformer (Ib) was favoured over (Ia) by the equatorial arrangement of the groups, and gave only the ring-contracted aldehyde compound. No epoxide, originating from Ia, was found. The cis compound (II) gave both the aldehyde and the ketone in almost equal amounts.



FIFURE X. Nitrous acid deamination of semipinacolic systems of aminocyclohexanols

The reaction of cis-2-amino-1-phenylcyclohexanol with nitrous acid resulted in 98.8% alkyl migration with ring contraction. In this study, Curtin and Schmuckler (1951) [17] concluded that the phenyl group had forced the amino group, respectively diazo group, into an equatorial position and there occurred no phenyl participation or migration, according to the first line of the mechanism shown in Figure XI. The carbonium ion intermediates of deamination were added by this author.

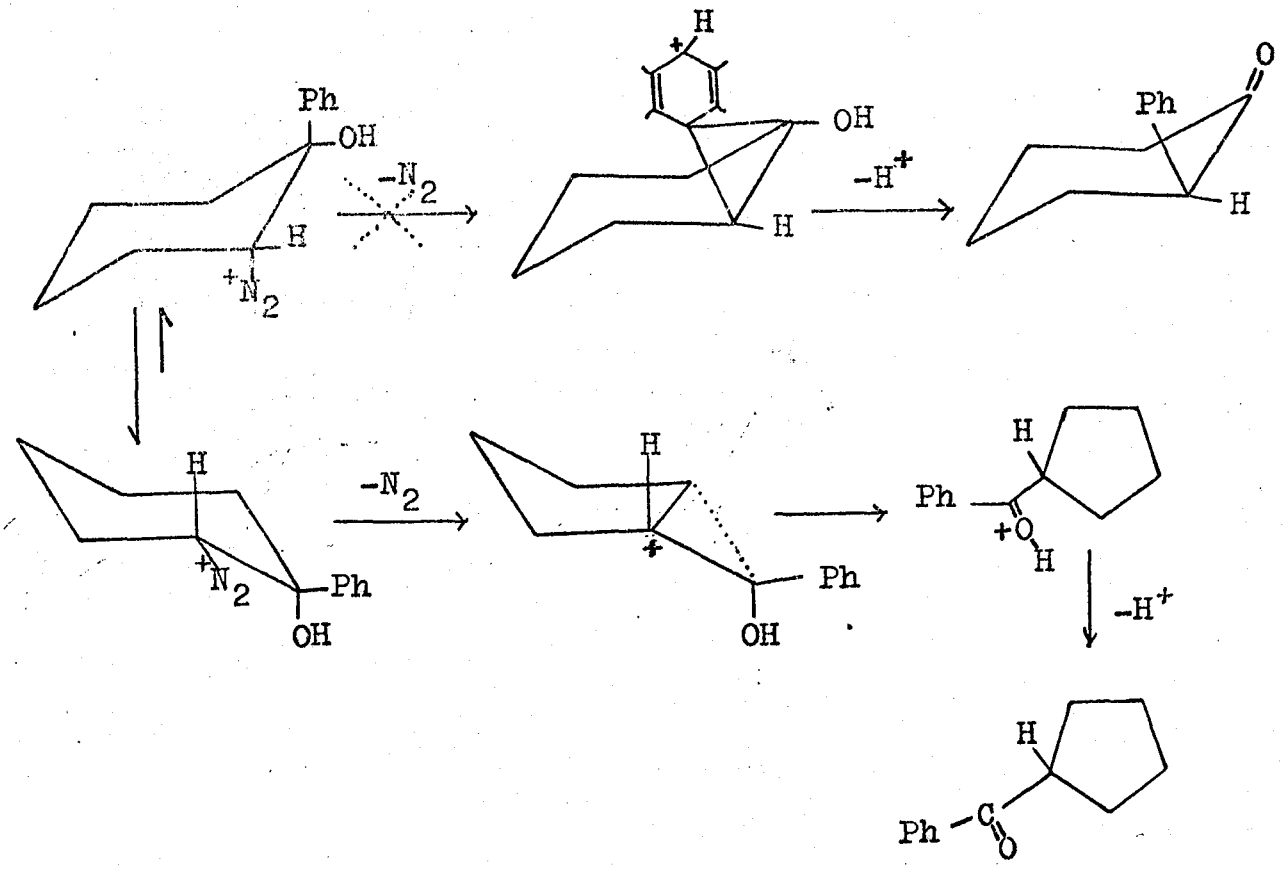


FIGURE XI. Carbonium ion intermediates of deamination of phenyl substituted amino cyclohexanol

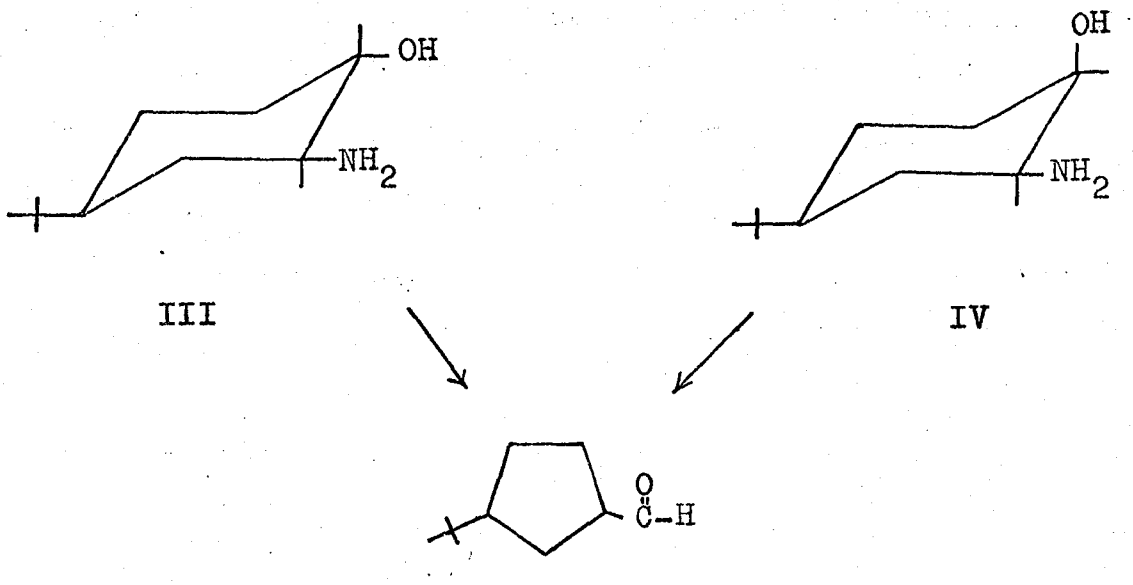
Cherest et al.[10] investigated the nitrous acid deamination of conformationally "fixed" 4-t-butyl-2-amino-cyclohexanols and compared their results to "mobile" systems, as summarized in the following table. They obtained three major products from their reactions.

Amino alcohol	Conformers		Total Yield	% Composition		
	$-NH_2$	-OH	%	Aldehyde	Ketone	Epoxide
Ia,b mobile,trans	{a e}	{a e}	89	99	trace	0
Ia,b mobile, cis	{a e}	{e a}	66	51-55	45-49	0
III fixed,trans	e	e	90	100	0	0
IV fixed, cis	e	a	98	100	0	0
V fixed, cis	a	e	76	2 - 3	97-98	0
VI fixed,trans	a	a	77	1 - 2	trace	98-99 "cis"epoxide

(mobile, referred to Figure X; fixed, referred to Figure XII)

TABLE I. Pentane-extractable deamination products of cyclic amino alcohols

These results clearly showed the anti-coplanar conformational requirement for the migrating groups. In "mobile" systems, the relative populations for the different conformational states would determine the relative proportions of the products. In "fixed" systems, there is only one conformation and less side-products are formed than in the "mobile" systems.



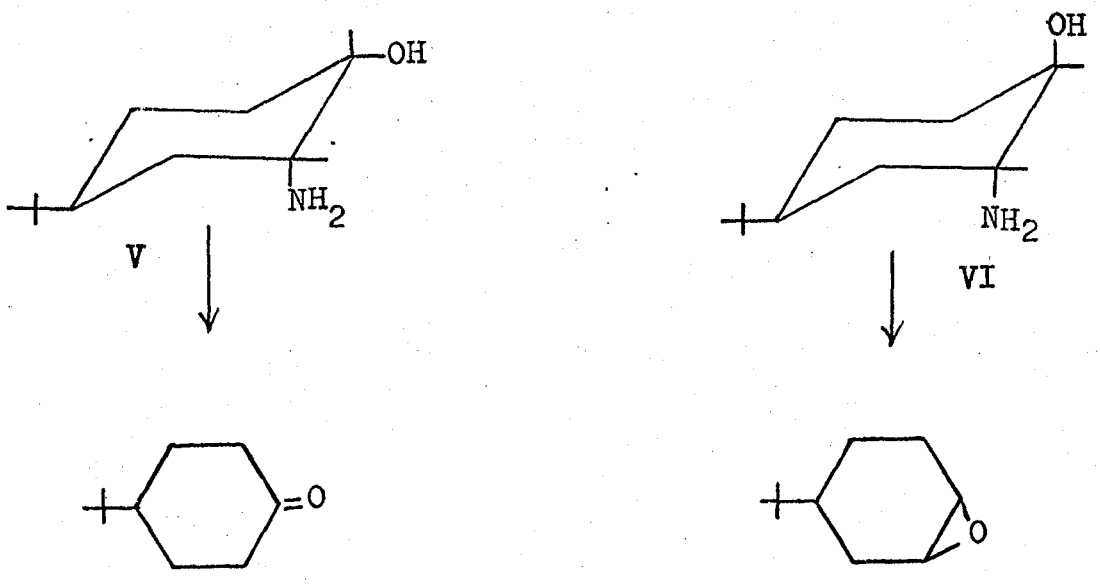


FIGURE XII. Nitrous acid deamination of epimers of 4-t-butyl-amino-cyclohexanols

DEAMINATION OF AMINO SUGARS

Open chain amino sugar derivatives generally follow the reaction patterns found in simple aliphatic analogues.

The nitrous acid deamination of 2-amino-2-deoxy-D-gluconic acid [62] gave 2,5-anhydro-D-gluconic acid with net retention of configuration and was analogous to the α-amino acid deaminations where double inversions caused net retention of configuration [5]. The mechanism is illustrated in Figure XIIa.

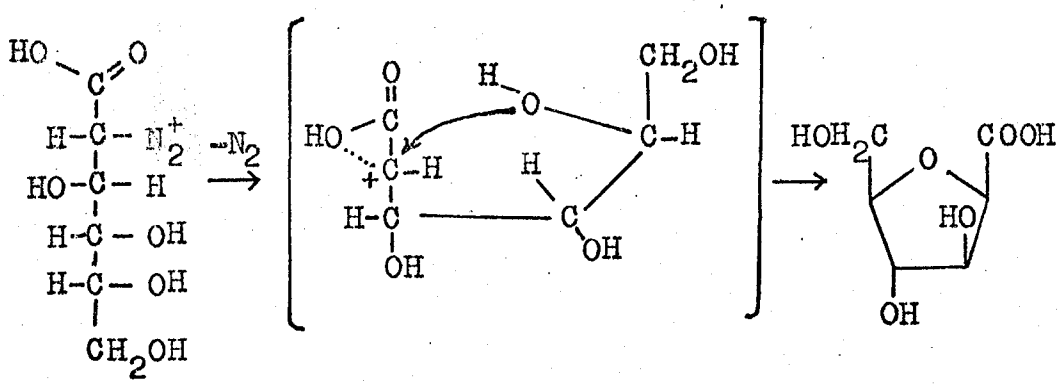


FIGURE XIIa. Deamination of 2-amino-2-deoxy-D-gluconic acid

It was shown by Matsushima [49] that the deamination of 2-amino-2-deoxy-D-glucitol gave 2-deoxy-D-arabino-hexose and the reaction was explained by Foster [24] as a normal carbonium ion reaction leading to olefin formation. The cation initially formed is stabilized by proton ejection to give the deoxy sugar in the enolic form which gives the aldehyde.

Free amino sugars are conformationally "mobile" cyclic systems. Deamination of D-glucosamine ("Chitosamine") with nitrous acid was first investigated by Ledderhose [43]. In the first detailed study of the problem, Fischer and Tiemann [23] showed that the deamination of "chitosamine" with nitrous acid gave a syrupy nitrogen-free reducing sugar. They oxidized the resulting "chitose" with nitric acid to form a monocarboxylic acid - "chitonic" acid [68] which was further oxidized to a dicarboxylic acid - "isosaccharic acid" [69]. From the findings of Tiemann [68, 69], and Fischer and Andrae [25a], an 2,5-anhydro-ring was assigned to "chitose". In a detailed series of investigations on the properties of related 2,5-anhydro-hexaric acids [45] evidence was presented for the assignment of a D-mannose configuration of "chitose" which could be reduced to crystalline 2,5-anhydro-D-mannitol.

Although 2,5-anhydro-D-mannose itself could not be crystallized, crystalline derivatives, such as a (diphenyl)-hydrazone [47] and a 2,4-dinitrophenylhydrazone [28b] of "chitose" were prepared.

The nitrous acid deamination of 2-amino-2-deoxy-D-glucose probably follows a concerted mechanism, the diazonium intermediate undergoing neighboring group participation by the ring-oxygen as the diazo group leaves from C-2 as a nitrogen molecule. The ring-oxygen is in a favourable position for this displacement when the molecule is in the Cl conformation, as is evident in the Newman projection shown in Figure XIII.

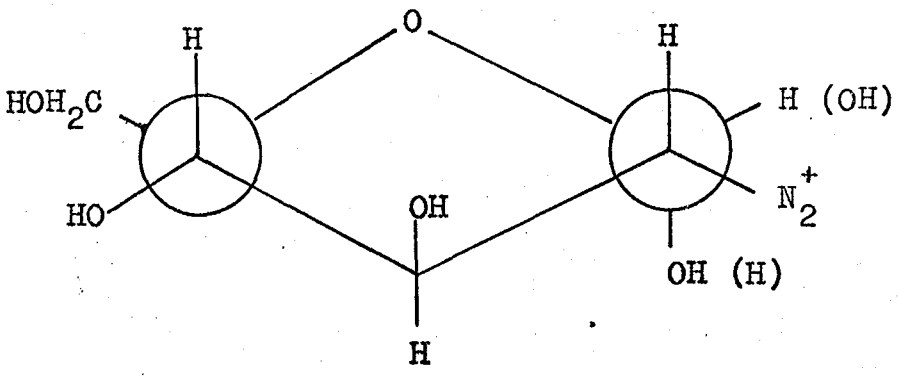
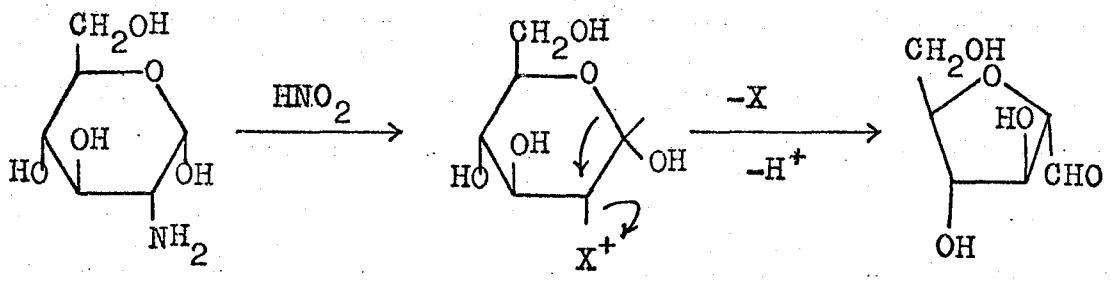


FIGURE XIII. Newman projection of a diazotized D-glucosamine

According to Peat [62], the displacement of the diazonium ion by the nucleophilic oxygen of the pyranose ring causes contraction of the ring (Figure XIV).



α-D-Glucosamine

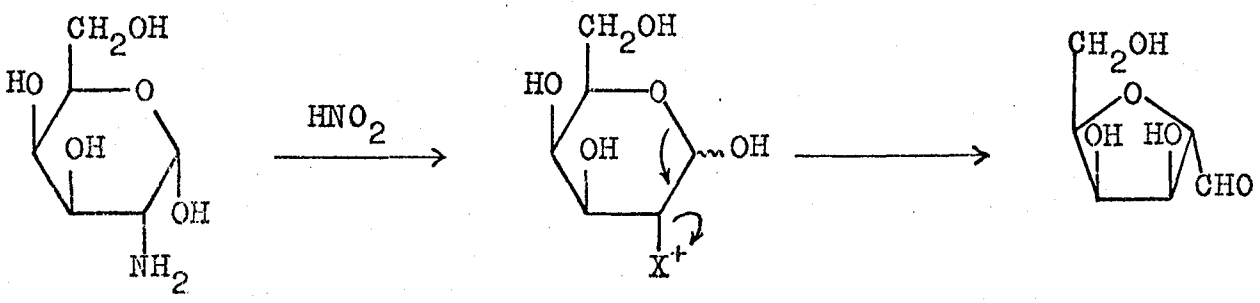
2,5-anhydro-D-mannose

$X^+ = N_2^+$ or $^+N_2OH_2$

FIGURE XIV. Deamination of α-D-glucosamine with nitrous acid

A glycosidic substituent, if present, is eliminated in the reaction, and the principal product is 2,5-anhydro-D-mannose. α-D-glycosides are deaminated more slowly than β-D-glycosides [2].

Similar to the deamination of D-glucosamine, 2-amino-2-deoxy-D-galactose (I), with nitrous acid, leads to 2,5-anhydro-D-talose (II), as shown in Figure XV. The nitrous acid deamination reaction as a degradation process was used for structural proof of a polysaccharide, containing 2-amino-2-deoxy-D-galactose, from Pneumococcus [1].



(I) (II)

$X^+ = N_2^+$ or $^+N_2H_2O$

FIGURE XV. Deamination of α-D-galactosamine with nitrous acid

2-amino-2-deoxy-D-mannose, with an axial amino group, upon nitrous acid deamination, was shown to give mostly D-glucose characterized as D-glucaric acid after oxidation with nitric acid [44].

However, the deamination of 2-amino-2-deoxy-D-mannose with mercuric oxide led to 2,5-anhydro-D-glucose [44]. At elevated temperature, the two chair conformers are apparently readily convertible and the surface deamination on mercuric oxide takes place through the less stable conformation (Ib) with an equatorial amino group, as shown in Figure XVI. The energy barrier for the formation of the less stable conformer in this reaction is probably small compared to the activation energy of the reaction, so that the less stable conformer can react.

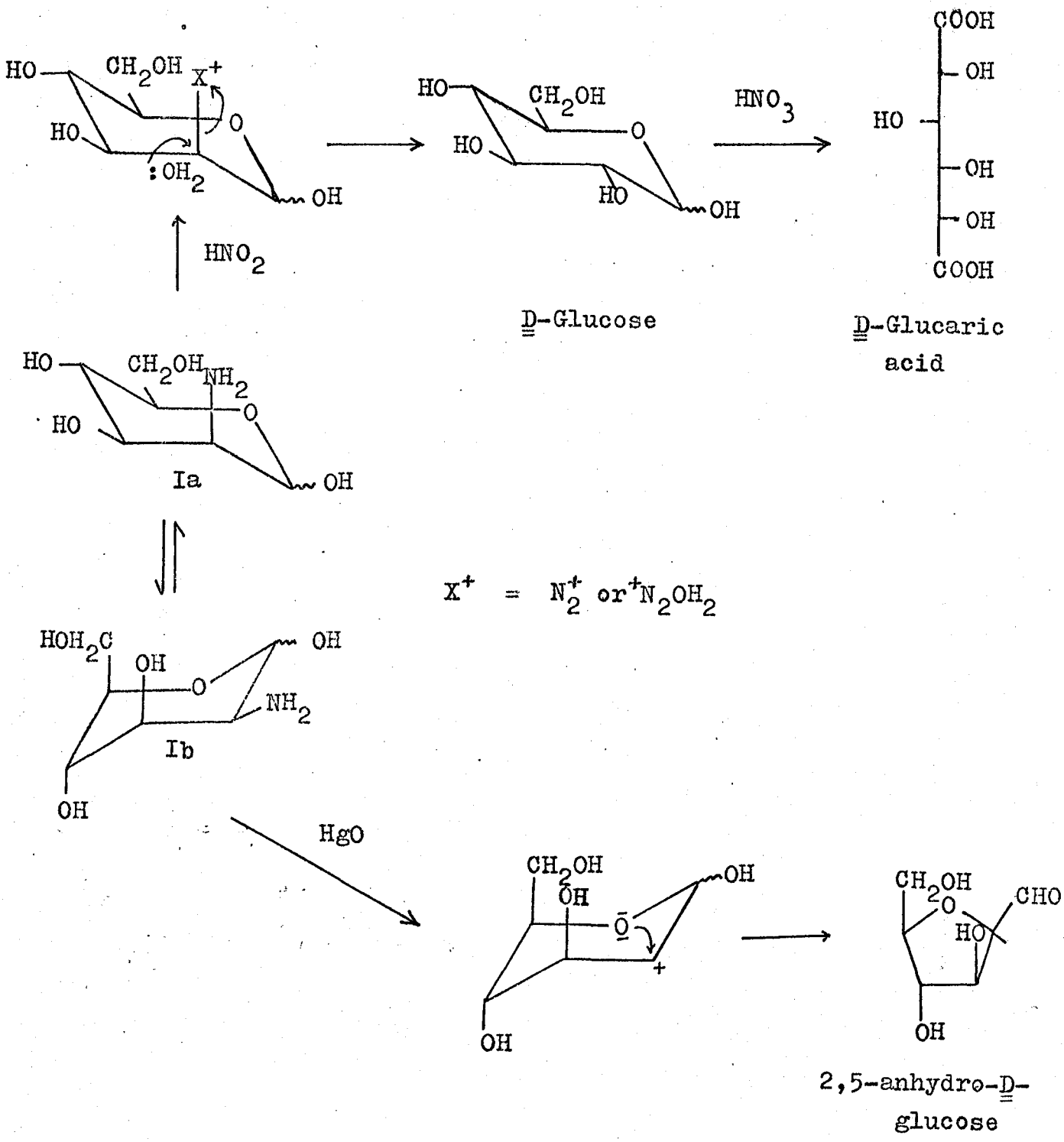


FIGURE XVI. Deamination of D-mannosamine

The deamination of 3-amino-3-deoxy- -D-altropyranose yielded a syrupy mixture of mannose and some 1,2-anhydro compounds which could not be identified [3].

Glycosides with fused ring systems that fix the pyranose ring in the C1 conformation, react according to the principles found by McCasland [50], Cherest [10], and Mills [51]. Reaction of methyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-altropyranoside (I), with nitrous acid, gave a quantitative yield of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside (II) [71].

Similarly, methyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (III) gave methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV) on treatment with nitrous acid. The amino and hydroxyl groups were fixed in axial and anti-parallel positions at C-2 and C-3 positions. The hydroxyl group came in from the rear as the N_2 group departed at the front, and epoxides were formed [71].

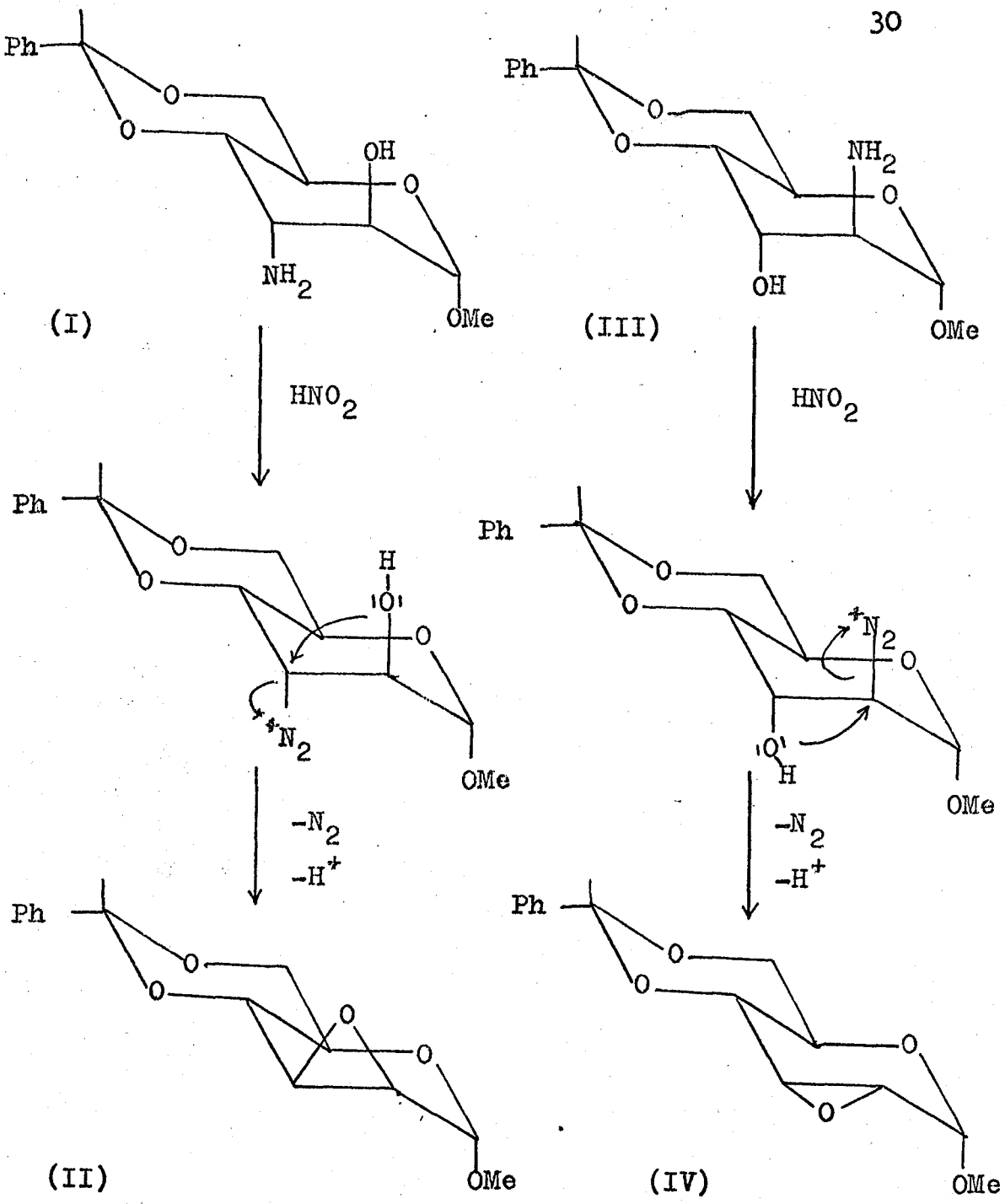


FIGURE XVII. Deamination of conformationally rigid amino-deoxy-α-D-altropyranoside with nitrous acid

Osawa and Akiya (1959) [61] found that the only isolable product of nitrous acid deamination of methyl 2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (a) was 2,5-anhydro-4,6-O-benzylidene-D-mannose (b). This ring contraction obeyed the conformational requirements for deamination as mentioned above.

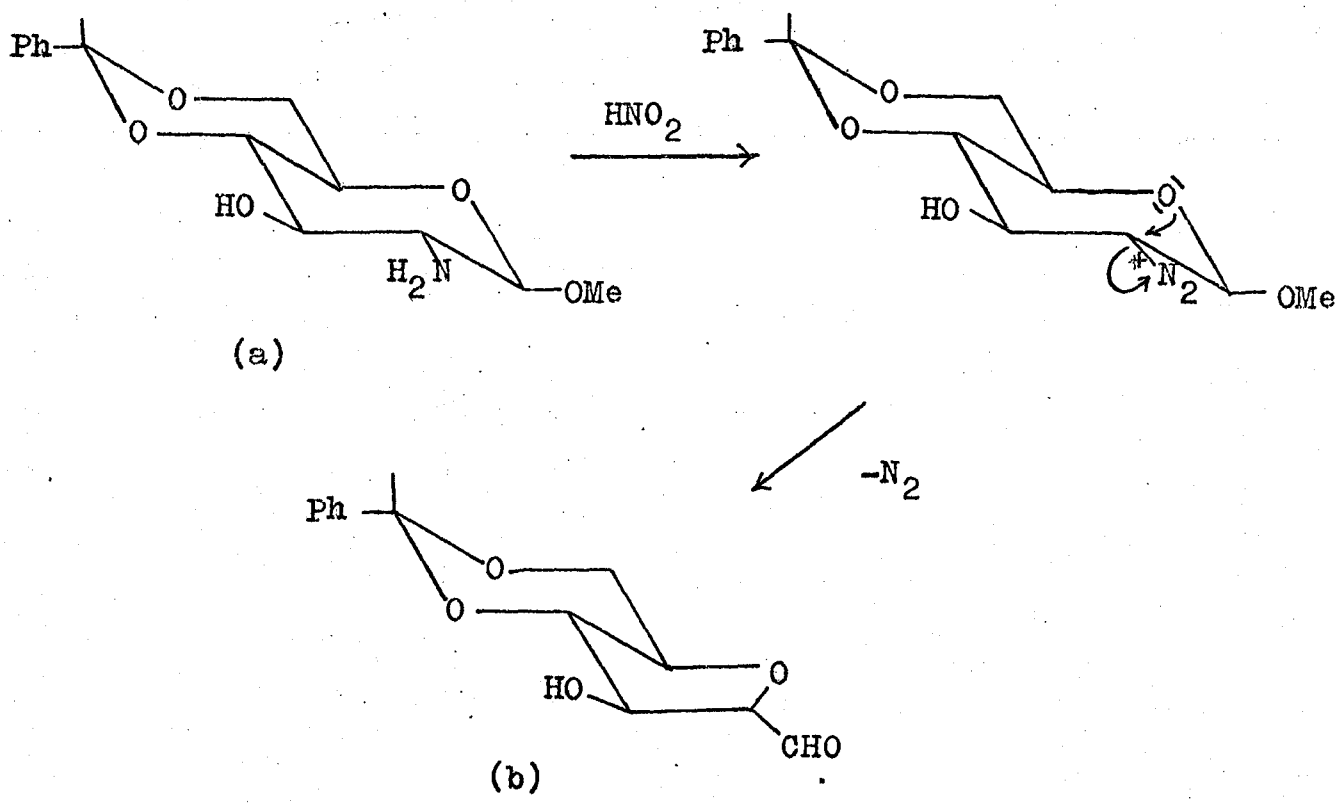


FIGURE XVIII. Deamination of methyl 2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside with nitrous acid

PROPOSED DEAMINATION STUDIES OF FUSED AMINO SUGAR DERIVATIVES

From the review presented here, it is apparent that work on conformationally restricted 2-amino-2-deoxy-D-hexose systems is scarce and such work has only been done on methyl glycosides. Work in our laboratory has centered on benzyl glycosides, which often have improved crystallization and solubility properties, that may allow isolation of small amounts from complex product mixtures. In addition, the bulkier benzyl groups may alter conformational preferences and change the course of reactions (Gross and Johnson, 1973) [29].

Deaminations of three benzyl 2-amino-4,6-O-benzylidene-2-deoxy-D-hexopyranosides were chosen for this investigation. The α-D-altropyranoside compound with a trans diaxial position of hydroxyl and amino groups was chosen as a case for which very little deviation from previous work was expected.

No work had been done on a conformationally fixed α-D-mannopyranoside compound. The competition between elimination involving C-2 and C-3, and anchimeric assistance of the α-benzyloxy group accompanying the deamination appeared to be of interest.

Finally, a comparison between the conformationally rigid α-D-gluco and β-D-gluco compounds was expected to give some additional insight into the deamination mechanism for equatorial amino groups.

CHAPTER II

PREPARATION OF STARTING MATERIALS

The author has prepared some of the starting materials for this study. The synthetic routes leading to the formation of one 3-amino and three 2-amino monosaccharide derivatives are discussed briefly in this chapter.

The homogeneity of the products from each reaction was determined by thin layer chromatography with different solvent systems. The choice of the system was based on the number of polar functional groups present in the molecule. Molecules with more polar groups would run slower in non-polar solvent. A more polar compound could thus be separated from a less polar compound with a suitable binary mixture of polar and non-polar solvents, such as methanol and chloroform. Mixtures of three solvents were used as needed in different cases in this study.

IR spectroscopy of pellets of potassium bromide mixed with the compound was used to identify specific functional groups. NMR Spectra were used to identify the key protons of the molecule. Optical rotations and melting points served to further characterize the products.

Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII) was prepared from D-glucose as shown in Figure XIX, according to the method of Chiu [11]. In the process, a new epoxide (V) and a new amino sugar derivative (VIII) were isolated as minor products.

Crude benzyl α-D-glucopyranoside, obtained from D-glucose and benzyl alcohol, was condensed with benzaldehyde/$ZnCl_2$ to give benzyl 4,6-O-benzylidene-α-D-glucopyranoside (II) subsequently mesylated into benzyl 4,6-O-benzylidene-2,3-di-O-methanesulfonyl-α-D-glucopyranoside (III). With alkali, two 2,3-anhydro-α-D-glycosides were obtained: benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV) and benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside (V). The latter, not found by Chiu, is only a minor product constituting 2% of the final yield. On treatment with potassium hydroxide in aqueous methanol, the manno-epoxide (V) gave a minor slow-moving spot and a major fast-moving spot on thin layer chromatograms. The minor product was isolated by thick layer chromatography and identified as the benzyl 4,6-O-benzylidene-α-D-altropyranoside (VI). The fast-moving

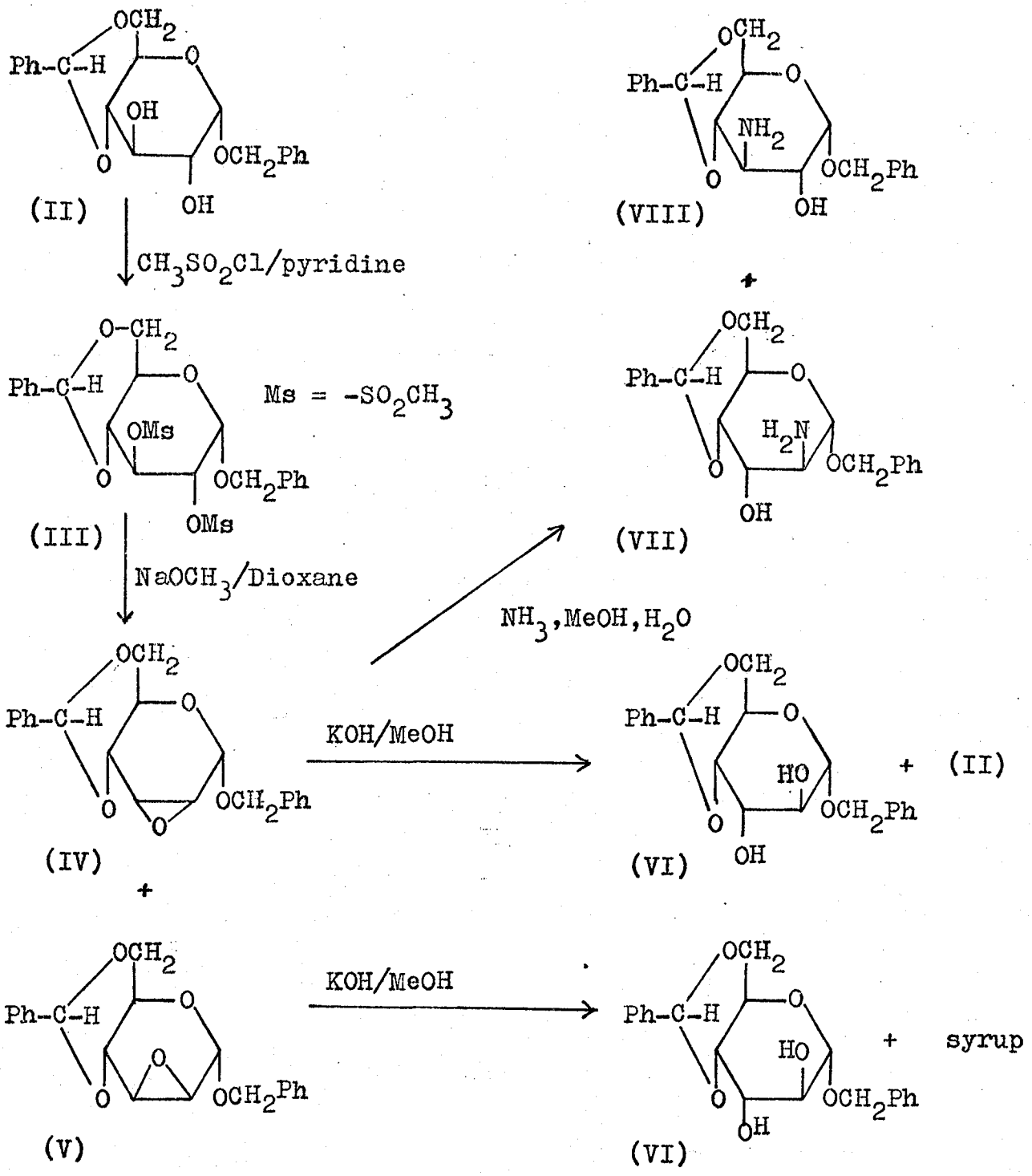


FIGURE XIX. Synthetic route to benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII)

component remained in form of syrup. The same treatment was applied to the allo-epoxide (IV) to give two components: benzyl 4,6-O-benzylidene-α-D-altropyranoside (VI) and benzyl 4,6-O-benzylidene-α-D-glucopyranoside (II), identical to the products that were already obtained in the same manner by Johnson [39].

Nucleophilic cleavages of epoxides obey the Furst-Plattner rule: "Trans diaxial opening is favoured over trans diequatorial opening in sterically fixed systems". Thus, the altroside compound was found to dominate over the glucoside compound in this reaction. The structure of the major product of the cleavage of the manno-epoxide (V) is not known.

When the allo-epoxide (IV) was reacted with ammonia in an autoclave for one or two days as described by Chiu [11], two amino sugars were obtained: benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII) and benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII). Also, in this case, the trans diaxial product, compound VII, predominates over the trans diequatorial product VIII.

The conversion of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII) into the α-D-mannopyranoside analogue (XII), was carried out in the manner described by Chiu

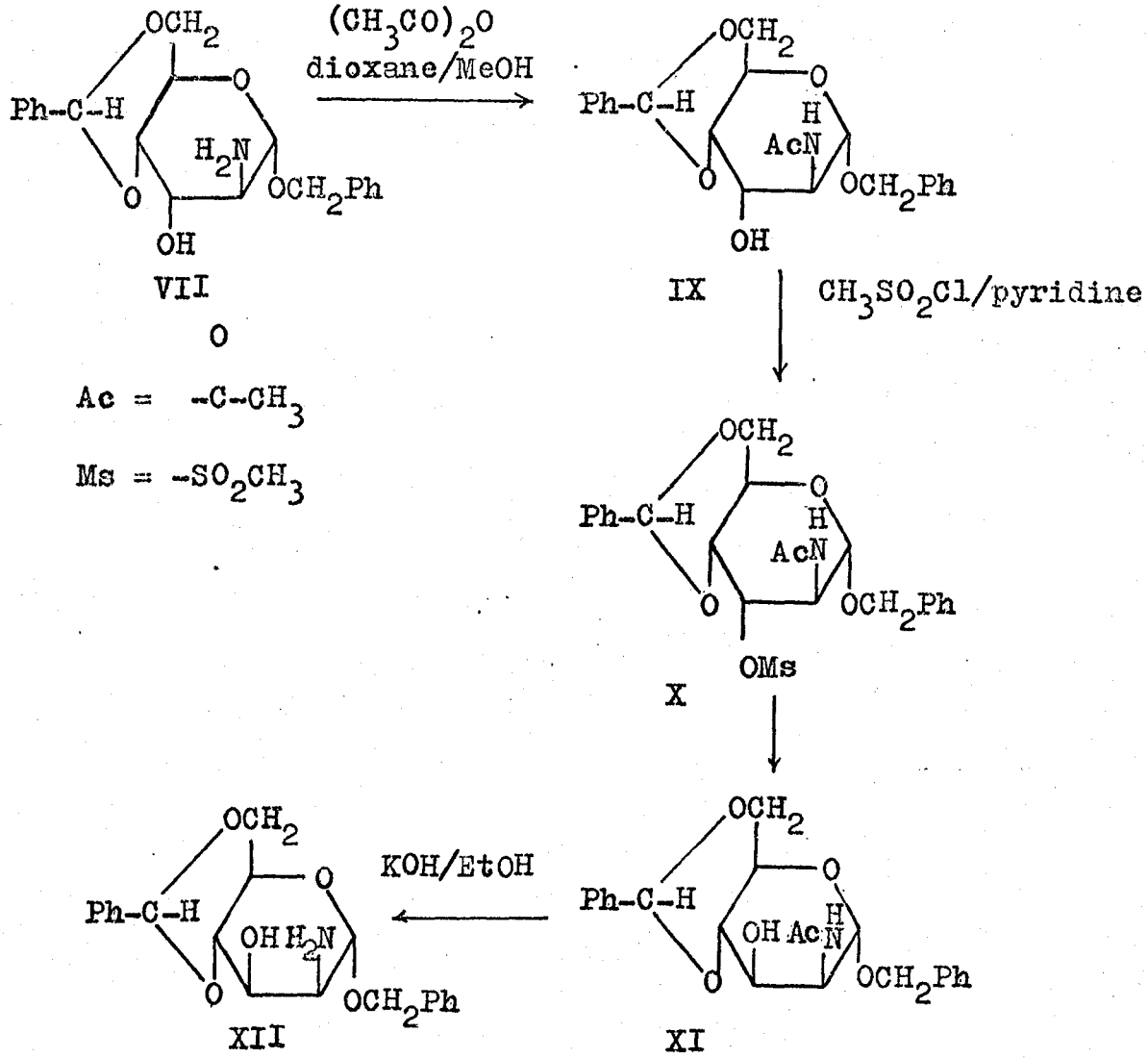


FIGURE XX. Synthetic route to benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-mannopyranoside (XII)

Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (XXIIa) prepared from 2-acetamido-2-deoxy-D-glucose [30], was obtained from our laboratory stock. The corresponding benzyl 2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (XXIIb), was prepared by the author according to published procedures as illustrated in Figure XXI. The peracetylated α-glycosyl chloride (XVIII) was obtained by treatment of 2-acetamido-2-deoxy-D-glucopyranose (XVII) with acetyl chloride [32]. Compound XVIII, when reacted with benzyl alcohol and $Hg(CN)_2$ gave the peracetylated benzyl β-D-glycoside (XIX) subsequently de-O-acetylated with triethylamine in methanol/water to form XX in a modification of the method by Kuhn and Kirschenlohr [42a]. From XX, the 4,6-O-benzylidene derivative XXI was prepared by treatment with benzaldehyde/$ZnCl_2$, followed by de-N-acetylation, to give XXIIb [30].

The benzyl amino-4,6-O-benzylidene-deoxy-hexopyranosides VII, VIII, XII, XXIIa and XXIIb were used in the nitrous acid deamination reactions discussed in the next chapter.

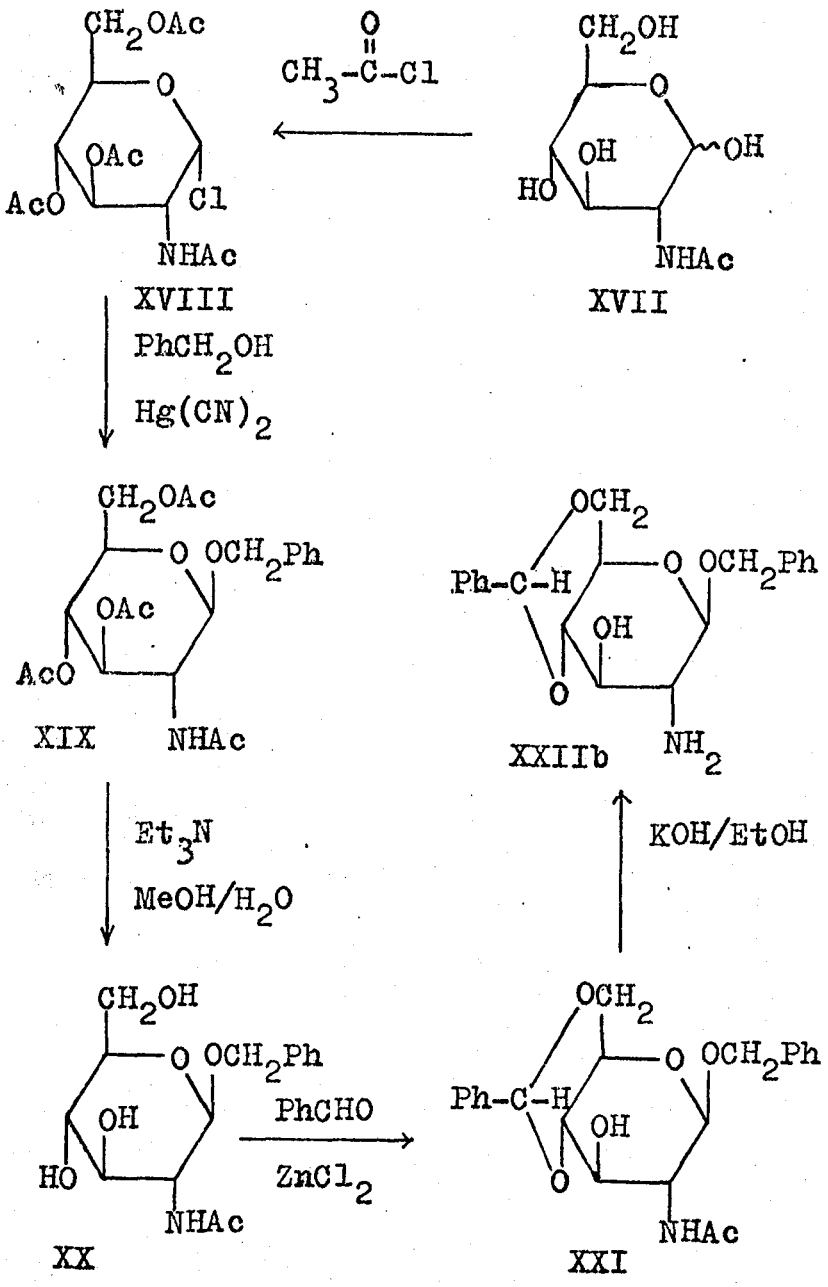


FIGURE XXI. Synthesis of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (XXIIb)

CHAPTER III

DISCUSSION OF METHODS AND RESULTS OF DEAMINATION REACTIONS

As major products from the deamination reactions, ketones, aldehydes or epoxides were anticipated. However, due to neighboring group participation, other products have also been observed. The benzyl glycosides in this thesis were found to be different from the methyl glycosides, in the reactions with nitrous acid.

The products of deamination reactions have been purified either by recrystallization or by thick layer chromatography separation. Due to difficulty in the separation of complex product mixtures, or due to difficulty in purification of oily products, some products were ignored in this study.

Intermediary cyclic oxonium ions are postulated to explain the formation of some of the observed deamination products. However, such intermediates were not isolated. The formation of diazonium ions from amino group and nitrous acid is assumed to follow the pattern suggested by Ingold [33-37].

The leaving of nitrogen from this intermediate, in the reactions observed in this study, can always be thought to have occurred in a concerted manner, either in an E_2-elimination process or with backside neighboring group participation by oxygen.

I. DEAMINATION OF BENZYL 2-AMINO-4,6-O-BENZYLIDENE-2-DEOXY-α-D-ALTROPYRANOSIDE (VII)

This reaction gave a quantitative yield of benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV). The result complied with the findings of Cherest et al.[10] that trans diaxial aminohydrin deamination gives epoxides. A corresponding epoxide is also formed from the methyl glycoside analogous to compound VII [71]. It is interesting to note, that these deaminations are simply reversals of the synthetic pathways which form the trans diaxial amino sugar derivatives from the epoxides by opening with ammonia [11].

The relationship is shown in Figure XXII. The fused cyclic acetal ring locks the sugar in the C1 conformation; the C-3 hydroxyl group is in a trans relationship to the leaving group - nitrogen. A carbonium ion formed

at C-2 is not stable and is attacked concertedly by the neighbouring hydroxyl from the backside to give an anhydro sugar.

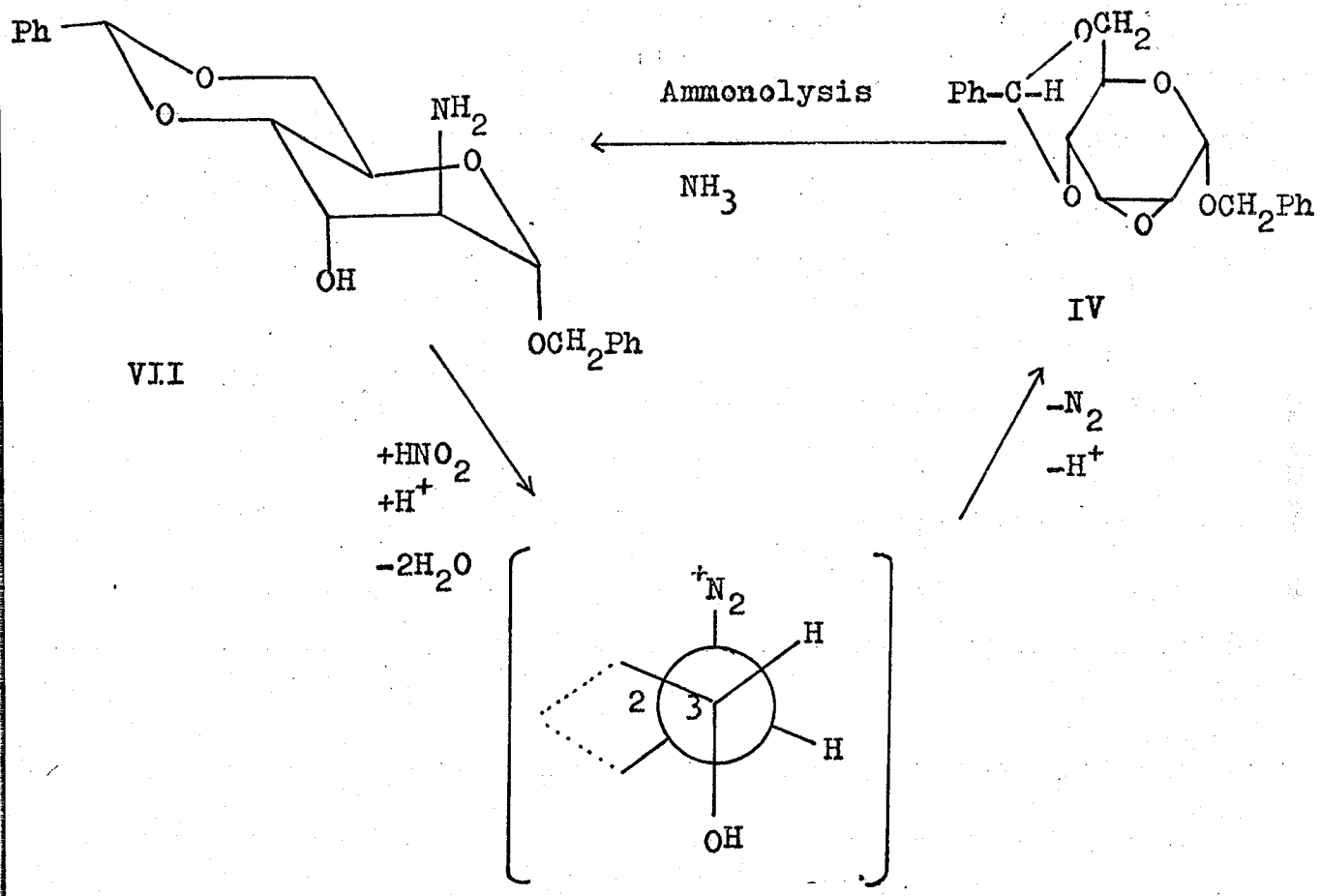


FIGURE XXII. Deamination reaction of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII)

II. DEAMINATION OF BENZYL 3-AMINO-4,6-O-BENZYLIDENE-3-DEOXY-α-D-GLUCOPYRANOSIDE (VIII)

Under the same condition as in the deamination of the altrosamine compound, benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII) also gave the same epoxide compound IV. However, there were many other side products which could not be isolated. Only the anhydro sugar (IV) was isolated as the fastest component by preparative tlc (Plate No. 4). The deamination reaction of VIII was expected to produce ring contraction to give benzyl 3-deoxy-3-C-formyl-α-D-xylofuranoside [38]. Yet, only the neighbouring group participation product was isolated in this case. Compared to the previous reaction, this reaction was much less specific.

The fixed C1 conformation makes the C-2 hydroxyl group gauche to the nitrogen leaving group in the Newman projection as in Figure XXIII. Yet, the hydroxyl group attacked the C-3 carbonium ion from the backside and formed an epoxide. It appears, that some deviation from the chair conformation is necessary to accomplish this. However, a

"mobile" 3-amino-3-deoxy-glucopyranoside compound did not give an epoxide and a ring contracted compound was isolated instead [38]. Normally, one would expect the "mobile" system to allow epoxide formation, and our "fixed" system to promote ring contraction.

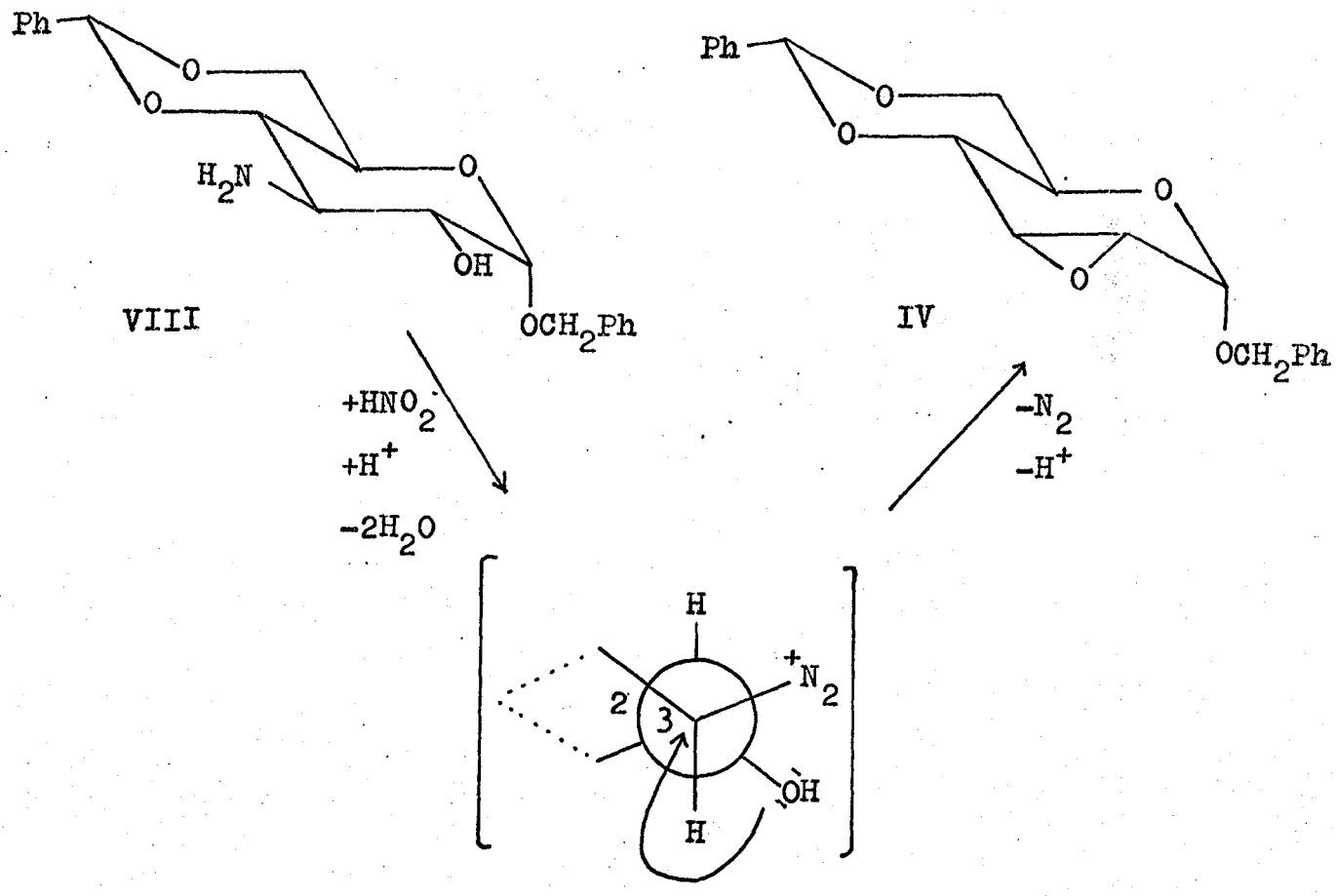


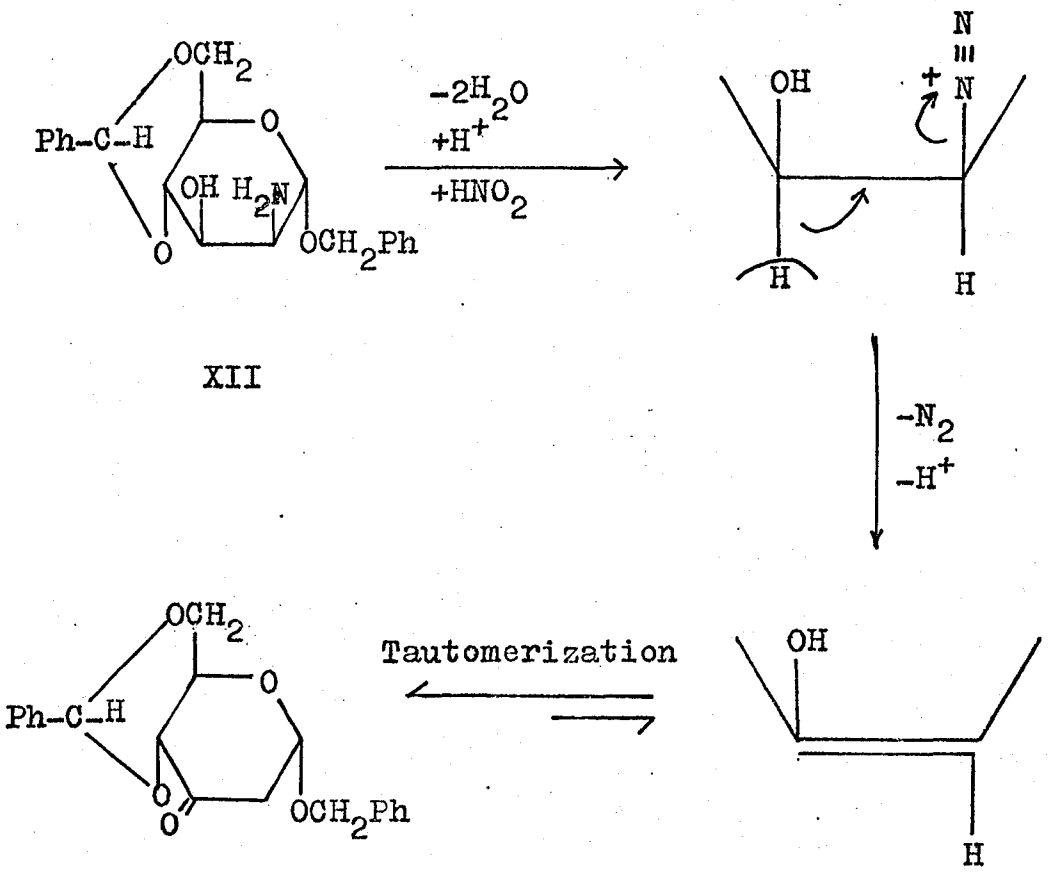
FIGURE XXIII. Deamination of benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII)

III. DEAMINATION OF BENZYL 2-AMINO-4,6-O-BENZYLIDENE-2-DEOXY-α-D-MANNOPYRANOSIDE (XII)

t-Butyl nitrite, first tried as the deamination reagent for this reaction, was found unsatisfactory. Too many side products were produced that were hard to separate by preparative tlc. The reaction was found to give a better result in a homogeneous reactant mixture at pH 5. Yet, the result was still in need of further improvement.

A citrate buffer solution of nitrous acid was finally used as the reaction medium mixed with a solution of amino sugar in dioxane. A pH 3.5 was found to be optimal. Compound XII gave only two major products: benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XIIIa) and 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV). The former was formed by elimination. A proton would leave from C-3 simultaneously with the nitrogen leaving at C-2 to give an enol. Tautomerization would favour the formation of the ketone tautomer as illustrated in Figure XXIV.

The ketosugar obtained from Tompkin's method [70], was found identical to compound XIIIa. This provided strong structural proof of the identity of XIIIa prepared by different routes.



XII

XIIIa

FIGURE XXIV. Formation of a ketone compound from the nitrous acid deamination of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-mannopyranoside (XII)

In competition, another mechanism appeared to operate in the deamination of XII, leading to the formation of a 2-O-benzyl sugar. The C-1 α-benzyloxy group participated in the nitrogen displacement by backside oxygen attack and formation of a cyclic oxonium ion. Such an oxonium ion would not be stable and the C-1 oxygen bond could be expected to cleave with backside attack of water. This would lead to the migration of the α-benzyloxy group from the C-1 axial position to a more preferable equatorial C-2 position as illustrated in Figure XXV.

The elimination product (XIIIa) was found to dominate over the benzyloxy migrated compound (XIV) in the mixture of deamination products of compound XII. The possible formation of the carbonium ion at C-2 after the loss of nitrogen leads to the competition of two pathways: elimination and benzyloxy migration. The former pathway appears to be preferred over the migration pathway, which is probably more sterically hindered. Both the C-3 hydrogen and C-1 benzyloxy groups are in the same anti-coplanar position relative to the C-2 nitrogen leaving group. The result complies with the findings of Cherest et al.[10] .

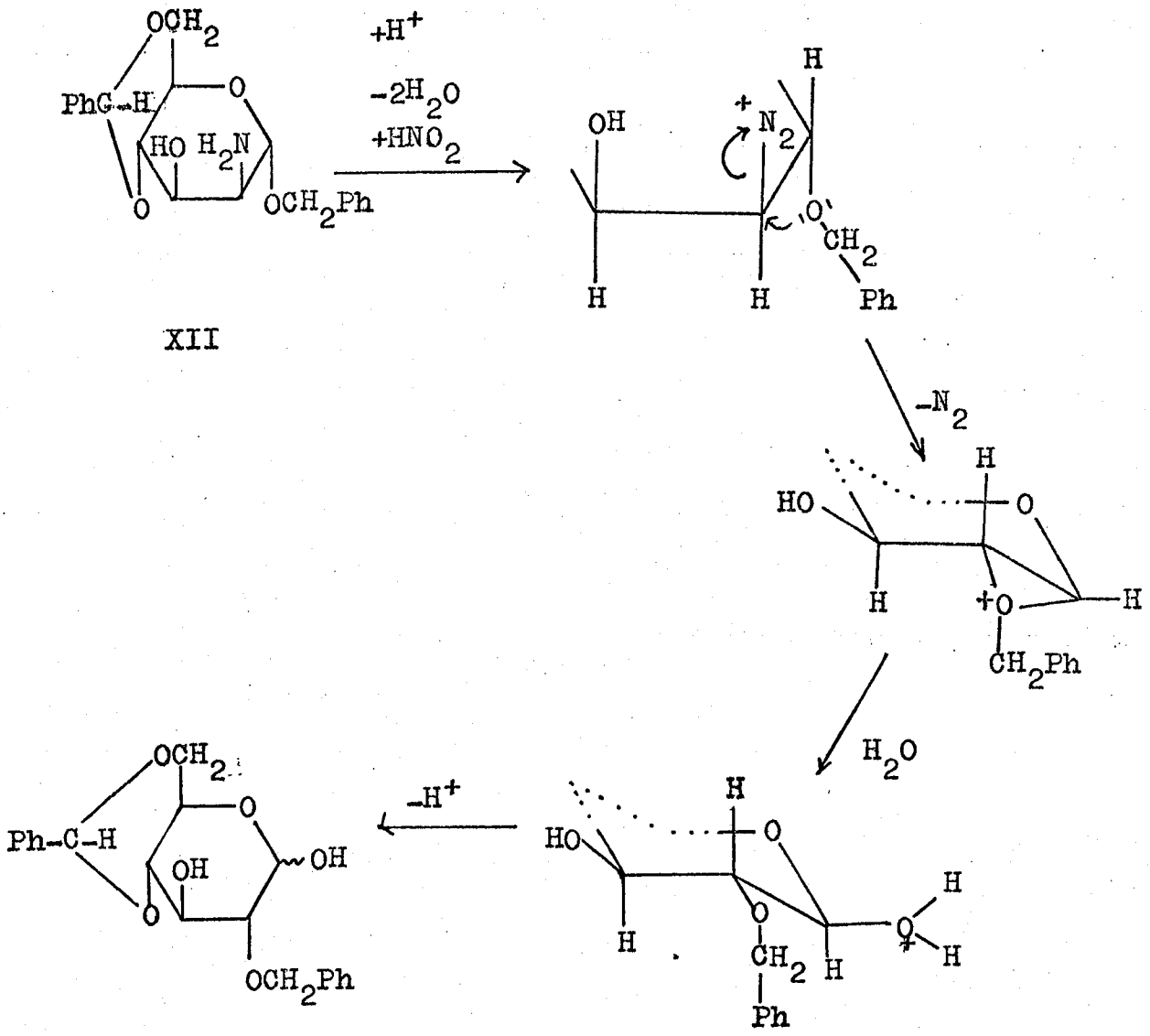


FIGURE XXV. Migration of a benzyloxy group in the deamination of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-mannopyranoside (XII)

The α-ketosugar was further treated with acetic acid and water to give a de-O-benzylidenated compound XV. The bath temperature and the duration of the reaction was critical. Decomposition was observed with temperatures above 73° or with reaction time longer than 30 minutes.

The benzylidene group is very sensitive to acid. Protons attack the acetal oxygen atoms. The resulting oxonium ions cleave between oxygen and the benzylic carbon to produce, ultimately, benzaldehyde.

The same method was used for the de-O-benzylidenation of 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV). The conditions were slightly varied to give a better yield. The resulting compound showed decreased optical rotation values after 24 hours, which indicated that the solid compound was the α-anomer. The IR spectrum was found to be identical to that of the authentic sample sent by Klemer [42] for comparison.

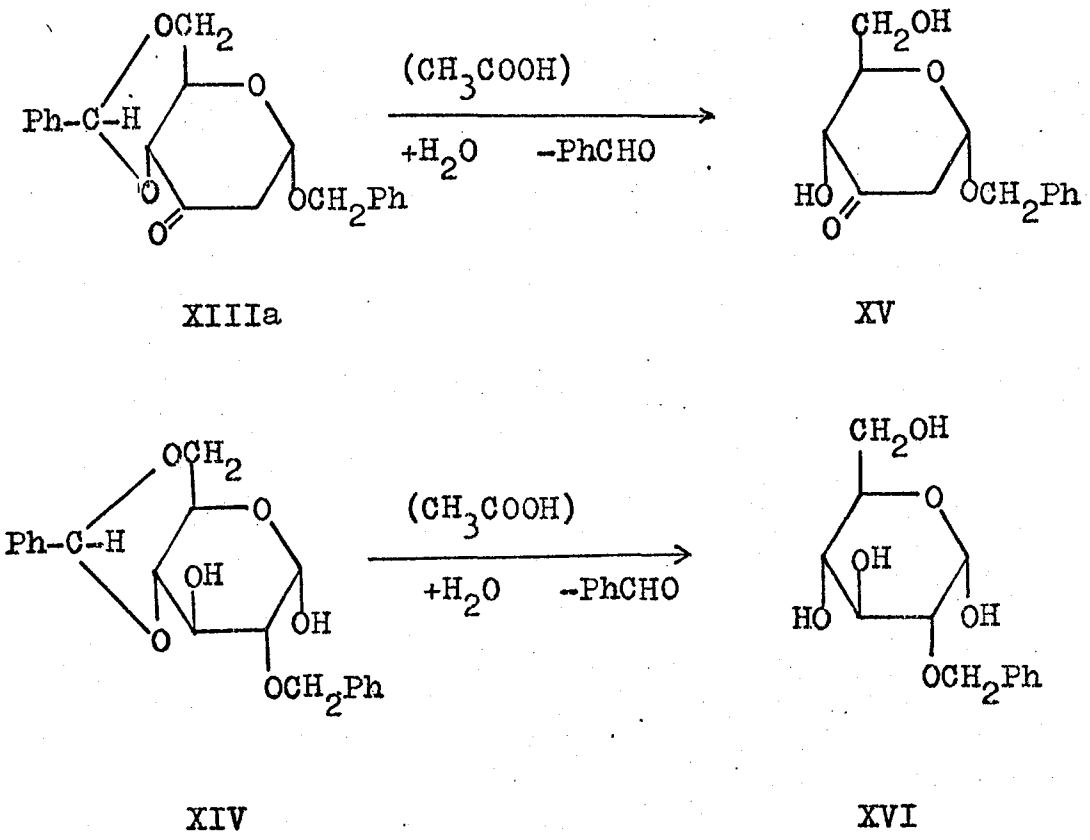


FIGURE XXVI. De-O-benzylidenation of XIIIa and XIV with acetic acid/water

IV. DEAMINATION OF BENZYL 2-AMINO-4,6-O-BENZYLIDENE-2-DEOXY-α-D-GLUCOPYRANOSIDE (XXIIa) AND β-D-GLUCOPYRANOSIDE (XXIIb)

Nitrous acid deamination of "mobile" glucosamine has been reviewed in Chapter I. The product was reported to be a ring-contracted 2,5-anhydro-D-mannose. The formation of 2,5-anhydro-4,6-O-benzylidene-α-D-mannose from the deamination of the "fixed" methyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside was also reported by Akiya and Osawa [61]. However, in the study presented in this thesis, the deamination of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (XXIIa) gave products, of which two were identical to those obtained from benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-mannopyranoside (XI). No compound with a contracted ring was isolated. The ketone compound (XIIIa) and the 2-O-benzyl compound (XIV) were isolated from a mixture of deamination products, by tlc analysis. In compound XXIIa, the amino and hydroxyl groups were fixed in a trans diequatorial gauche position by the benzylidene ring. Such conformation does not obey the requirement of anti-coplanar relationship for neighboring group participation to give elimination or benzyl migration product, However,

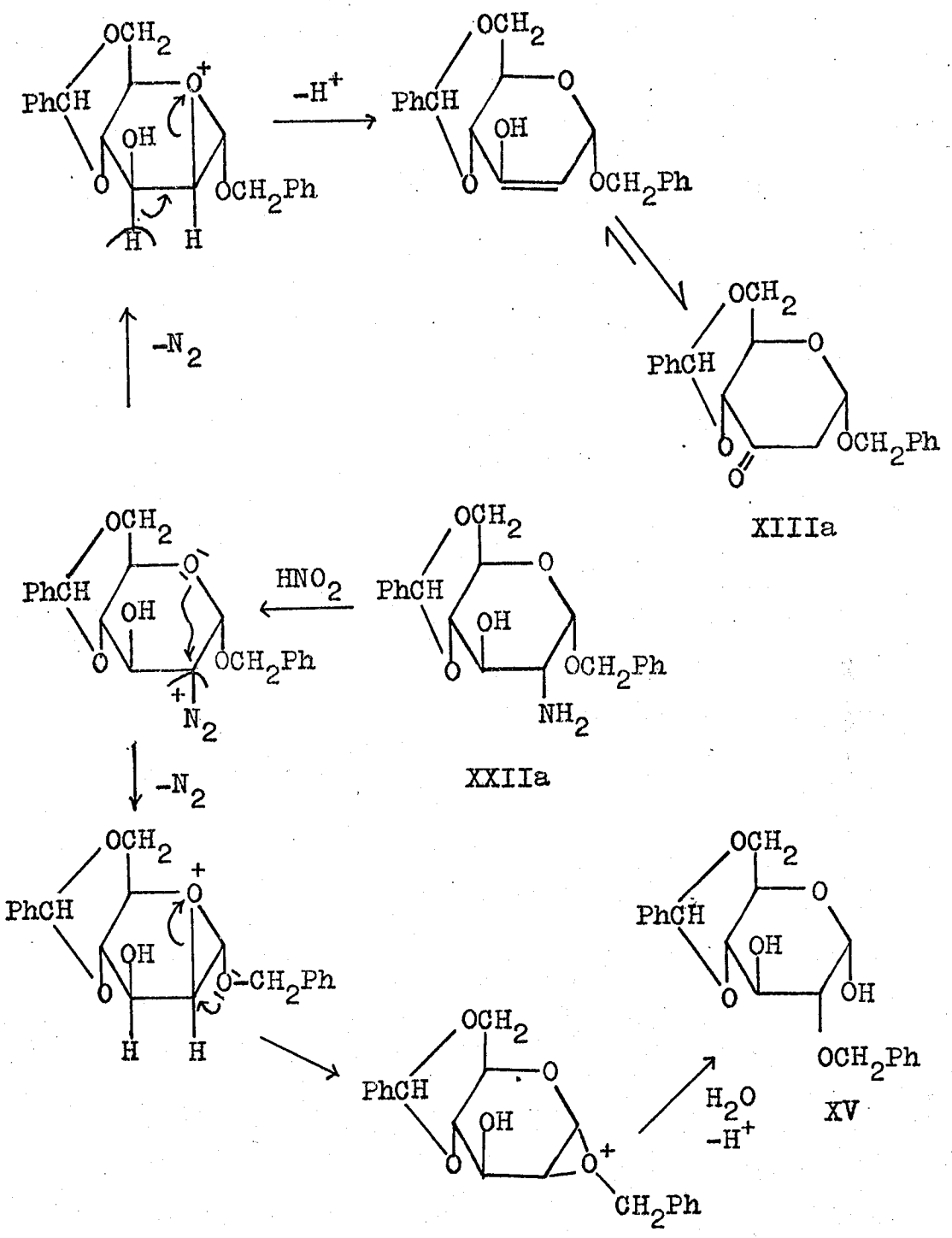
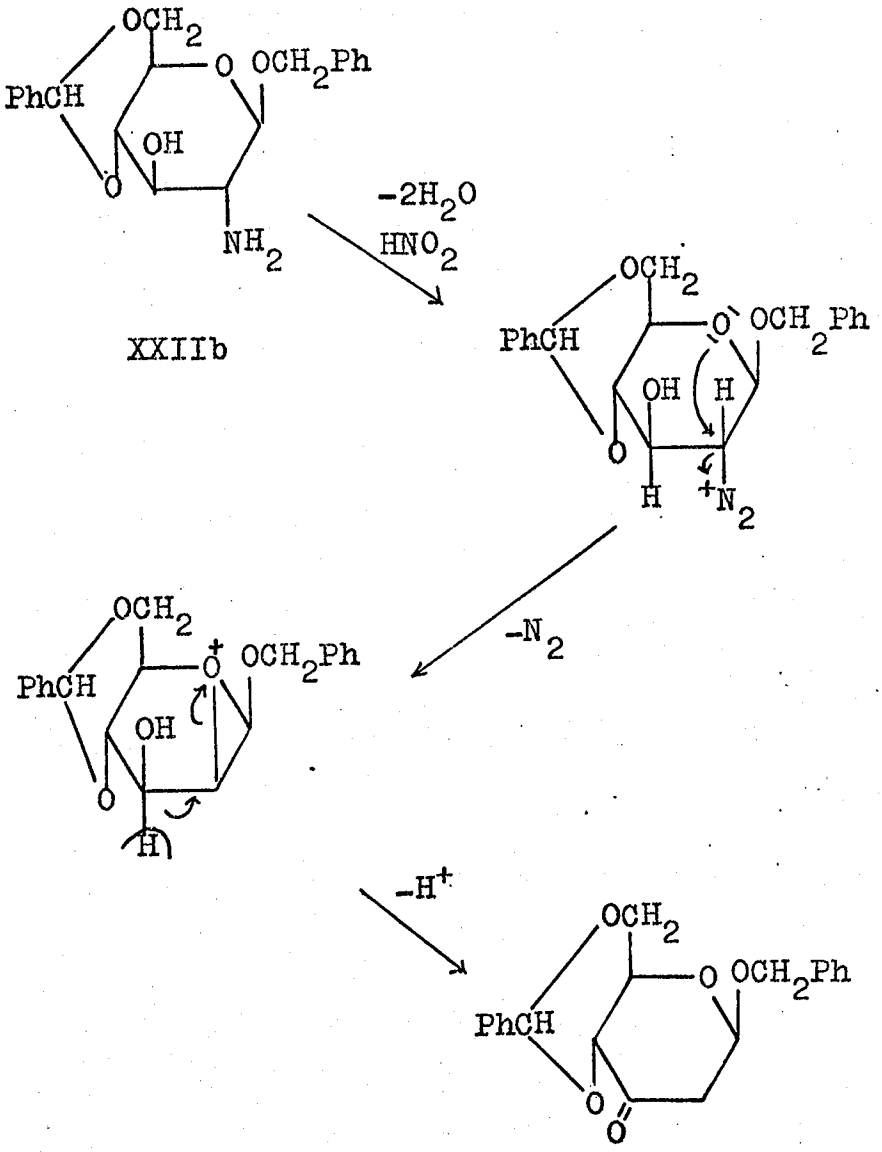


FIGURE XXVII. Deamination of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (XXIIa)

the pyranose ring oxygen would be in the position to assist the nitrogen displacement with backside attack that would lead to the formation of a bicyclic oxonium ion. This bicyclic oxonium ion, having actually a D-manno configuration, is then believed to give the same products as the mannosamine derivative XI. The scheme of reactions is summarised above in Figure XXVII.

The deamination of the β-glucopyranoside amino sugar (XXIIb) gave also a ketone compound: benzyl 4,6-O-benzylidene-2-deoxy-β-D-erythro-hexopyranosid-3-ulose (XIIIb). However, the formation of a 2-O-benzyl compound from the intermediate bicyclic oxonium ion is here sterically impossible. The IR spectrum of compound XIIIb was very similar to that of the α-anomer compound XIIIa. Melting points were different but XIIIa and XIIIb behaved very similarly in tlc analysis. The elemental analysis results of these α- and β-ketosugars were identical. The author was unable to isolate other compounds from the mixture of the deamination products of the β-anomer (XXIIb) because most of these components ran very closely to each other on the preparative tlc plates and were hard to separate.



XIIIb

FIGURE XXVIII. Deamination of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (XXIIb)

From the products obtained from the deamination of these benzyl amino-4,6-O-benzylidene-hexopyranosides: VII, VIII, XII, XXIIa and XXIIb, it is concluded that the anti-coplanar relationship of groups participating in a deamination reaction is not absolutely required. However, amino sugars for which such a relationship could be realized gave less complex product mixtures.

Hydrogenation of the benzyl α-D-erythro-hexopyranosid-3-ulose (XV) with palladium/charcoal as catalyst to give a free ketosugar was attempted. The objective was to cleave selectively the benzyl group by hydrogenation, while the ketone function was to be retained. Water was used in the medium with the hope to hydrate the ketone function to prevent it from being reduced. After one equivalent of hydrogen was used up, the resulting compound was soluble in water and crystallized from isopropanol. Further characterization is necessary, however.

Similar ketosugars, such as 2-keto-3-deoxy-D-glucose, have been isolated from soybean and from arsenite treated liver cells of mice [21]. These compounds were thought to be involved in metabolic regulation and also in growth

control [19]. Experiments were conducted in fed and fasted mouse livers to observe the release of such keto-aldehyde compounds after treatment with As_2O_3. The result showed the possibility that glycogen or glucose when complexed to other substances such as amino acids, could be transformed by As_2O_3 to release a free keto-aldehyde [20]. It is interesting to note, that the identity of the isolated compound with a synthetic sample [20] of 2-keto-3-deoxy-D-glucose was never satisfactorily shown.

CHAPTER IV

SUMMARY AND CONCLUSION

A series of benzyl glycosides have been prepared as starting materials for the study of nitrous acid deamination reactions. During the preparation of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII), benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside (V) and benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII) were isolated as new compounds that were minor by-products of IV respectively VII. Alkaline cleavage of V gave the same altropyranoside (VI) which was obtainable from the alkaline cleavage of benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV).

Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII) and benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII) gave benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV) on nitrous acid deamination. Thus, the trans diaxial arrangement of -OH and $-NH_2$ groups does not appear to be an absolute requirement, although the reaction of the trans diaxial amino alcohol VII was more specific.

Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-mannopyranoside (XII) reacted with nitrous acid to give, by elimination, benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XIIIa) and, by α-benzyloxy migration, 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV). Compound XIIIa was then treated with aqueous acetic acid to give benzyl 2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XV). Similarly, compound XIV gave 2-O-benzyl-α-D-glucose (XVI).

Contrary to expectation, from the deamination of benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (XXIIa), the same products were isolated as from the deamination mixture of the α-D-manno analogue. Ring contraction products could not be isolated. A bicyclic oxonium ion is postulated as an intermediate in this reaction. Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (XXIIb), on treating with nitrous acid, gave benzyl 4,6-O-benzylidene-2-deoxy-β-D-erythro-hexopyranosid-3-ulose (XIIIb), analogous to the α-anomer, along with two other major unidentified oily compounds. A 2-O-benzyl derivative is not to be expected in this case. Thus, these compounds may contain ring contracted structures.

This investigation shows that product composition for deamination with our systems and blocking groups is very different from previously published results.

CHAPTER V

EXPERIMENTAL PROCEDURES

GENERAL

Melting points were taken in a Thomas-Hoover melting point apparatus Model No. 6404H. All melting points reported herein are uncorrected. Optical rotations were measured at the sodium D line with an O.C. Rudolph and Sons Inc. Model No. 956 polarimeter. Infrared spectra were recorded with a Perkin-Elmer spectrophotometer, Model 337, using the potassium bromide pellet technique with a Wilkes pellet compressor. The Nuclear Magnetic Resonance (NMR) spectra were obtained with a JEOL MINIMAR-100 100 MHz NMR spectrophotometer. The homogeneities of all compounds synthesized were determined by thin layer chromatography using a mixture of two parts Merck silica gel G with one part Merck silica GF_{254}, the plates being activated by heating at 120° for 2 hours. The plates were developed with chloroform containing lesser amount of ethanol, methanol or acetone. Different solvent systems were used for developing different compounds as would be indicated in separate procedures. The compounds were detected by extinction of the ultraviolet fluorescence of a zinc-silica indicator and

also by subsequent spraying with sulfuric acid (10%-15%)-methanol and heating for about 15 min. at 120°. If several compounds were found by tlc analysis, they were labeled A, B, C, etc. on the plates starting from the fastest, in the individual preparations. The preparative tlc separations were done on silica gels from Chemie-Erzeugnisse und Adsorptionstechnik AG, Schweiz, on layers of 0.75 mm. Elutions of silica gel fractions were done with the solvents indicated in the individual procedures, in vessels immersed in an ultrasonic bath. The microanalyses were performed by Beller Mikroanalytisches Laboratorium, Göttingen, West Germany. Buffer of pH 3.5 was made up for the deamination reactions by dissolving citric acid (12.5 g) and sodium hydroxide (2.0 g) in water (50 ml).

Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV)

a. A solution of benzyl 4,6-O-benzylidene-2,3-di-O-methanesulfonyl-α-D-glucopyranoside (III) [11] (30.0 g, 0.06 mol) and sodium methoxide (9.72 g, 0.18 mol) in absolute dioxane (360 ml) and methanol (50 ml) was stirred at 5°C for 5 hours and at room temperature for 6 days. The precipitate was filtered out and the filtrate was evaporated to dryness in vacuo at room temperature. Residue and precipitate were shaken with water for 5 hours. The solids were filtered out and recrystallized from absolute ethanol to give IV (16.5 g, 81%), mp 189°, with IR spectrum identical to the compound prepared by Chiu [11]. Literature [11]: mp 180-182°. The mother liquor of this recrystallization was used for the isolation of V.

b. Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII) (0.34 g, 1.0 mmol) dissolved in dioxane (12 ml), was mixed with a solution of citrate buffer (8 ml) and sodium nitrite (0.9 g, 13 mmol). The mixture was stirred overnight at room temperature. Saturated sodium bicarbonate solution was added to bring the pH to 7. The precipitate was filtered out and washed with water to give 0.3 g of crude product with only one major component by tlc analysis. Recrystallization from absolute ethanol gave IV (0.22 g, 65%), mp 191-192°, $[\alpha]_D^{23}$ +104° (C=1, pyridine), ν_{max} 1320, 1350 (epoxide); 700, 750 (C_6H_5). Literature [10]: mp 180-182°, $[\alpha]_D^{25}$ +105° (C=1, pyridine).

c. Benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII) (0.16 g, 0.0045 mol) dissolved in dioxane (5 ml) was added to a solution of sodium nitrite (0.3 g, 4.35 mmol) in citrate buffer (2.5 ml). The solution was stirred at room temperature. Saturated sodium bicarbonate solution was added to bring the pH to 7. White precipitate (0.05 g) was filtered out and washed with distilled water (10 ml). The filtrate was extracted three times with dichloromethane (3 X 35 ml). The extracts were evaporated

to give a residue (0.09 g). Both the precipitate and the residue showed three major components in tlc analysis with chloroform:benzene:methanol (85:25:10) (Plate No.4).

The fast moving component A (Plate No.4) was isolated by preparative tlc in the same solvent system. It was eluted by chloroform and recrystallized from absolute ethanol to give IV (0.05 g, 30%),mp 192°. The IR spectrum was found identical to that of the deamination product of VI in procedure "b".

Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside (V)

The mother liquor obtained from the procedure "a" of the preparation of IV was evaporated *in vacuo* to dryness to give 0.7 g of solid. After it was dissolved in warm methylcyclohexane, benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV) crystallized on cooling, and was filtered out. The filtrate was concentrated and applied to a preparative tlc plate. The fast running band, in the solvent system of carbon tetrachloride: chloroform (1:1), was eluted* by CH_3Cl. The residue from the eluate evaporated, was dried *in vacuo* recrystallized from carbon tetrachloride to give V (0.36 g, 2%), mp 128-129°, $[\alpha]_D^{23}$ +63° (C=1, pyridine), ν_{max} 1258, 830 (epoxide); 696, 732, 751 (C_6H_5). Anal. Calcd. for $C_{20}H_{20}O_5$ (340.36): C, 70.57; H, 5.92 Found: C, 70.93; H, 5.52.

* Plate No. 2

Benzyl 4,6-O-benzylidene-α-D-altropyranoside (VI)

Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside (V) (0.1 g, 0.0003 mol) was dissolved in methanol (2 ml) and KOH (2.5 g) in H_2O (2.5 ml) was added. The solution was heated in a sealed autoclave at 125° for 24 hours. A precipitate formed after addition of water. It was filtered and became gummy on the filter paper. Tlc analysis, with chloroform as solvent, showed three major components. The slow component C (Plate No.10) was isolated by preparative tlc and eluted with chloroform, to give, after evaporation, VI (0.01 g, 9%), mp 184°, ν_{max} 3450 (OH); 743, 700 (C_6H_5). Literature [11]: mp 183-4°, ν_{max} 743, 699 (C_6H_5). Compound VI and the sample obtained from Chiu [11] behave the same in tlc analysis.

Benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII)

Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV) (23.0 g, 0.068 mol) was heated with methanol saturated with ammonia (920 ml) and concentrated ammonium hydroxide solution (230 ml) in a 2-liter sealed stainless steel autoclave at 110° for 48 hours. From the clear solution, methanol was evaporated in vacuo and the precipitate was filtered out, dissolved in tetrahydrofuran, and reprecipitated with diisopropyl ether. Recrystallization from absolute ethanol gave VII [11], identical to the compound prepared by Chiu [11], by IR spectrum and melting point.

The filtrate from the reprecipitation (with THF/diisopropyl ether) was evaporated to give a dry residue. It was dissolved in boiling water, and the solution was decanted from impurities. Crystals formed on slow cooling and were recrystallized from a small amount of tetrahydrofuran to give VIII (0.4 g, 1.6%), mp 160-163°, mixed melting point with (VII) 140-150°, $[\alpha]_D^{25}$ +74° (C=0.7, pyridine), ν_{max} 3350, 3300 (NH); 695, 750 (C_6H_5). The IR spectrum was different from that of VII in the regions of 1300-1000 cm^{-1}, and 800-600 cm^{-1}. Anal. Calcd. for $C_{20}H_{23}NO_5$ (357.4): C, 67.20; H, 6.49; N, 3.92 Found: C, 67.21; H, 6.29; N, 3.81

Benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyransid-3-ulose (XIIIa)

a. Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-mannopyranoside (XII) (1.9 g, 5.3 mmol) dissolved in dioxane (70 ml) was added to a solution of sodium nitrite (2.5 g, 36.2 mmol) in citrate buffer (40 ml). The solution was stirred at room temperature for 18 hours. Saturated sodium bicarbonate solution was added to increase the pH to 7. The filtrate was evaporated to dryness in vacuo and the residue was shaken in distilled water (50 ml). The white precipitate (1.35 g) was filtered off and washed twice with distilled water (50 ml). The water phase was extracted with dichloromethane (3 X 35 ml). The extracts were evaporated in a rotary evaporator to give a residue (0.1 g).

The precipitate and the residue, by tlc analysis(Plate No.5), showed two major products. Isolation of the faster component by preparative tlc with chloroform:benzene:acetone (65:30:5), after elution with chloroform, gave XIIIa (0.85 g, 47%), mp 164-165°, $[\alpha]_D^{26}$ +105° (C=1, pyridine), ν_{max} 1740 (C=O); 690, 750 (C_6H_5). Literature [70]: mp 164-165°, $[\alpha]_D^{23}$ +106°, (C=1, pyridine), ν_{max} 1740 (C=O); 690, 750 (C_6H_5).

b. Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (XXIIa) (0.17 g, 0.5 mmol) dissolved in dioxane (6 ml) was added to a solution of sodium nitrite (0.46 g, 0.7 mmol) in citrate buffer (4 ml). The solution was stirred at room temperature overnight. Saturated potassium bicarbonate solution was added for neutralization. The filtrate was evaporated to dryness in vacuo and the residues were shaken in distilled water (15 ml). The white precipitate (0.1 g) was filtered out and washed with distilled water (5 ml). The water phase was extracted three times with dichloromethane (40 ml). The solvent was evaporated in vacuo to give a residue (0.03 g).

Both the precipitate and the residue, by tlc analysis, showed three major components. Isolation of the component B (Plate No.6) by preparative tlc with chloroform:benzene:acetone (65:30:5), after elution with chloroform, and crystallization from methylcyclohexane, gave XIIIa (0.05 g, 30%), mp 160-163°, identical to that of the deamination product of XI in the procedure "a" above.

Benzyl 4,6-O-benzylidene-2-deoxy-β-D-erythro-hexopyranosid-3-ulose (XIIIb)

Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (XXIIb) (0.36 g, 1.0 mmol), dissolved in dioxane (12 ml), was added to a solution of sodium nitrite (0.9 g, 1.4 mmol) in citrate buffer (8 ml). The solution was stirred at room temperature overnight. Saturated potassium bicarbonate solution was added for neutralization. The filtrate was evaporated to dryness in vacuo and the residues were shaken in distilled water (30 ml). The white precipitate (0.14 g) was filtered out and washed with distilled water (20 ml). The water phase was extracted with dichloromethane (3 X 35 ml). The extracts were evaporated in a rotary evaporator to give an oily residue (0.11 g).

Both the precipitate and the residue, by tlc analysis, showed three major components. Isolation of the fast component A (Plate No.7) by preparative tlc with chloroform:benzene:acetone (65:30:5), after elution with chloroform, and crystallization from methylcyclohexane, gave XIIIb (0.04 g, 11%), mp 183°, $[\alpha]_D^{26}$ -74.1° (C=0.54, dichloromethane), ν_{max} 1740 (C=O), 685, 730, 750 (C_6H_5). Anal.Calcd. for $C_{20}H_{20}O_5$ (340.36): C, 70.57; H, 5.92 Found: C, 69.98; H, 5.93

2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV)

a. From the tlc separation of XIIIa in procedure "a", the slow component B (Plate No. 5) was eluted with chloroform and recrystallized from chloroform/methylcyclohexane to give XIV (0.5 g, 26%), mp 175°, $[\alpha]_D^{24}$ +150° (5 min)→ +6° (1 hr and 4 hr), (C=1, pyridine), ν_{max} 3425 (OH); 700, 750 (C_6H_5). Anal. Calcd. for $C_{20}H_{22}O_6$ (358.4): C, 67.02; H, 6.19 Found: C, 67.17; C, 6.24

b. From the tlc separation of XIIIa in procedure "b", the slow component C (Plate No.6) was eluted with chloroform and recrystallized from chloroform/methylcyclohexane to give XIV (0.02 g, 11%), mp 175°, $[\alpha]_D^{24}$ +140° (5 min)→ +10° (1 hr and 4 hr). The IR spectrum was identical to that of the product obtained in procedure "a" above.

Benzyl 2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XV)

Benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XIIIa) (0.17 g, 0.5 mmol) was heated in glacial acetic acid (6.0 ml) and water (0.1 ml) at 73° for 30 minutes. Water (4 ml) was added and the solvents were evaporated in vacuo at a bath temperature below 50°. More water (4 ml) was added, and evaporated in vacuo, followed by methanol (4 ml) and toluene (4 ml) with subsequent evaporations until the residue was dried. It was recrystallized from hot methylcyclohexane to give XV (0.09 g, 71.4%), mp 82.5-83.5°, $[\alpha]_D^{24}$ +128.5° (C=1, methanol), ν_{max} 1720 (C=O); 695, 740 (C_6H_5); 3350, 1280 (OH). Anal. Calcd. for $C_{13}H_{16}O_5$ (252.29): C, 61.77; H, 6.38 Found: C, 61.60; H, 6.51

2-O-benzyl-α-D-glucose (XVI)

2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV) (0.1 g, 0.27 mmol) was dissolved in glacial acetic (4 ml) and water (2 ml) was added. The solution was heated to 76° for 2 hours. Water (4 ml) was then added and the solvents were evaporated in vacuo at a bath temperature below 50°. More water (4 ml) was added, evaporated in vacuo, followed by methanol (4 ml), and toluene (4 ml), with subsequent evaporations until the residue was dried. The compound was recrystallized from hot methylcyclohexane to give XVI (0.07 g, 93%), mp 176-177°, $[\alpha]_D^{26}$ +61° (5 min) $\longrightarrow$ +45° (24 hr) (C=1, methanol), ν_{max} 3475, 1275 (OH); 750, 700 (C_6H_5). Literature [41]: mp 176-177°, $[\alpha]_D^{23}$ +56° (15 min) $\longrightarrow$ +47° (24 hr) (C=1, methanol). The IR spectrum was identical to that of the authentic sample obtained from Klemer [42].

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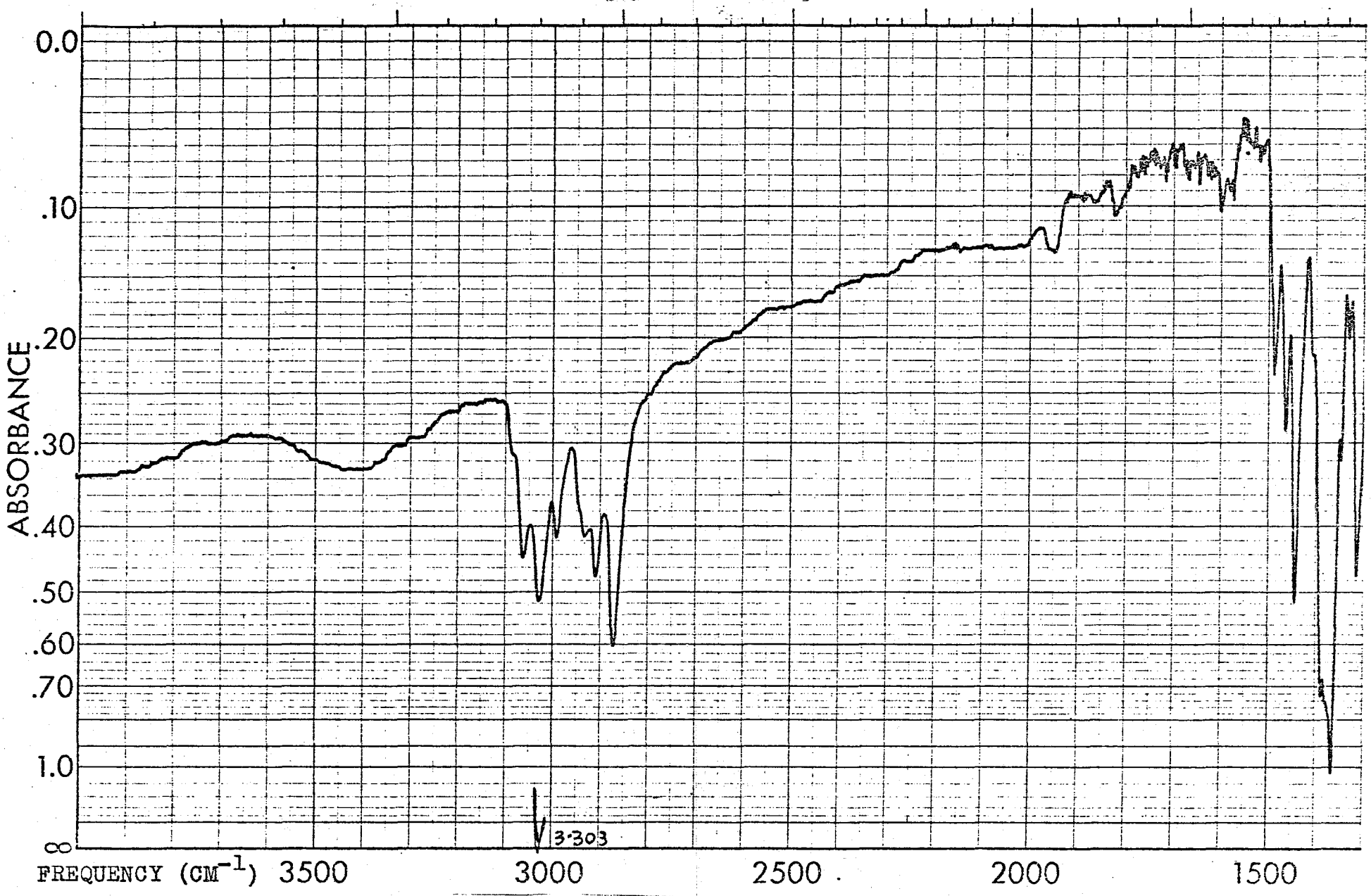

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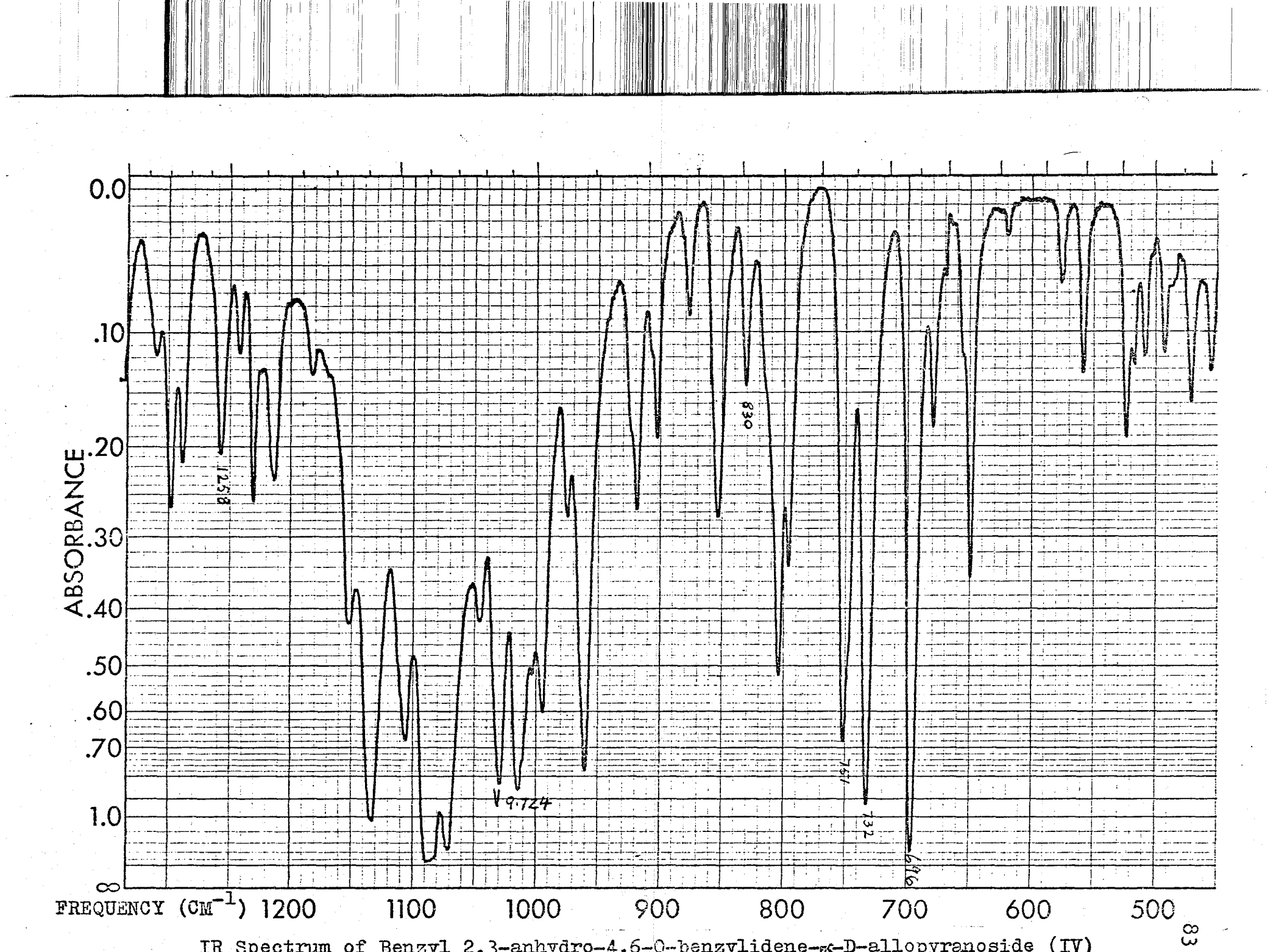

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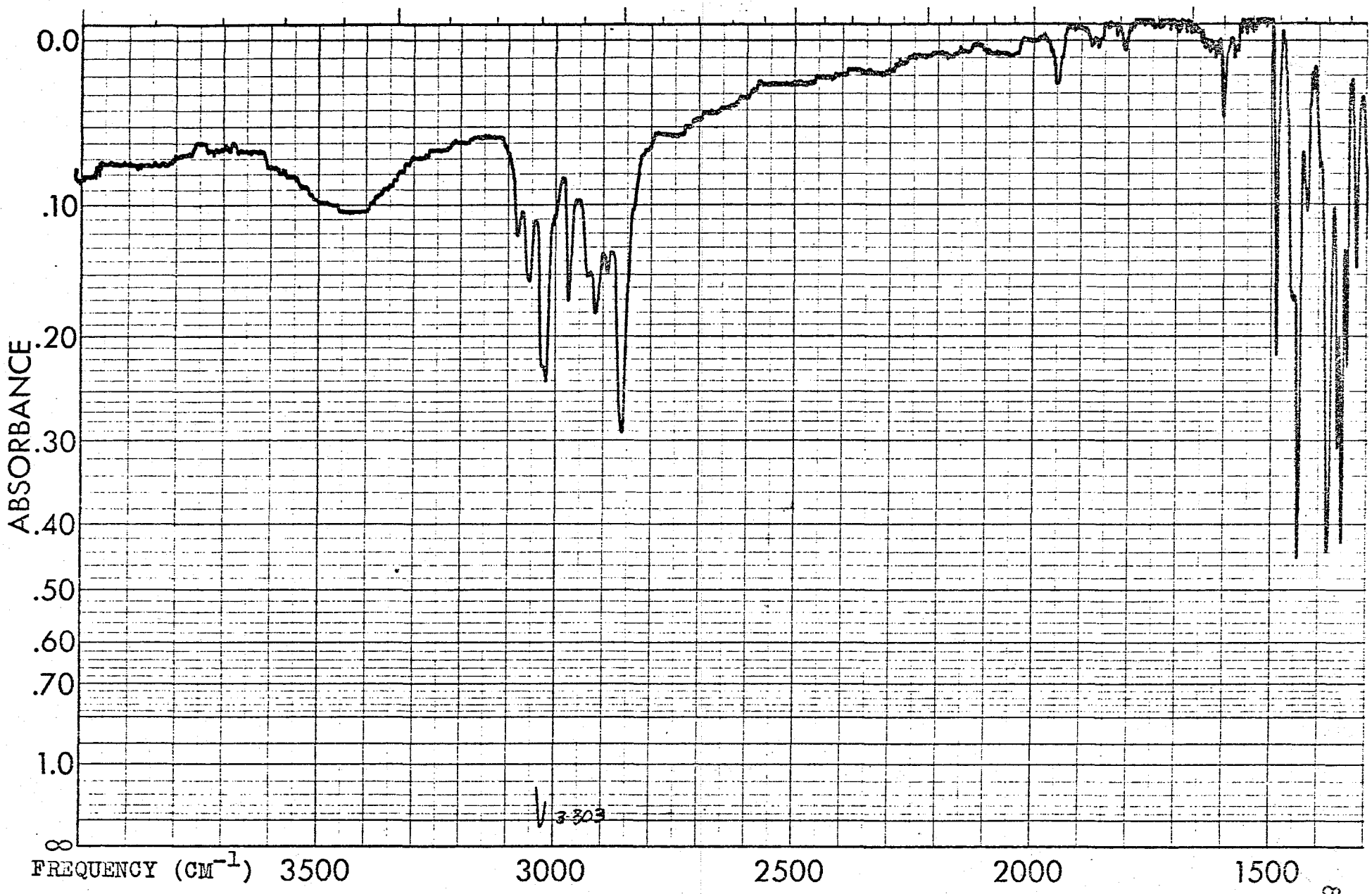

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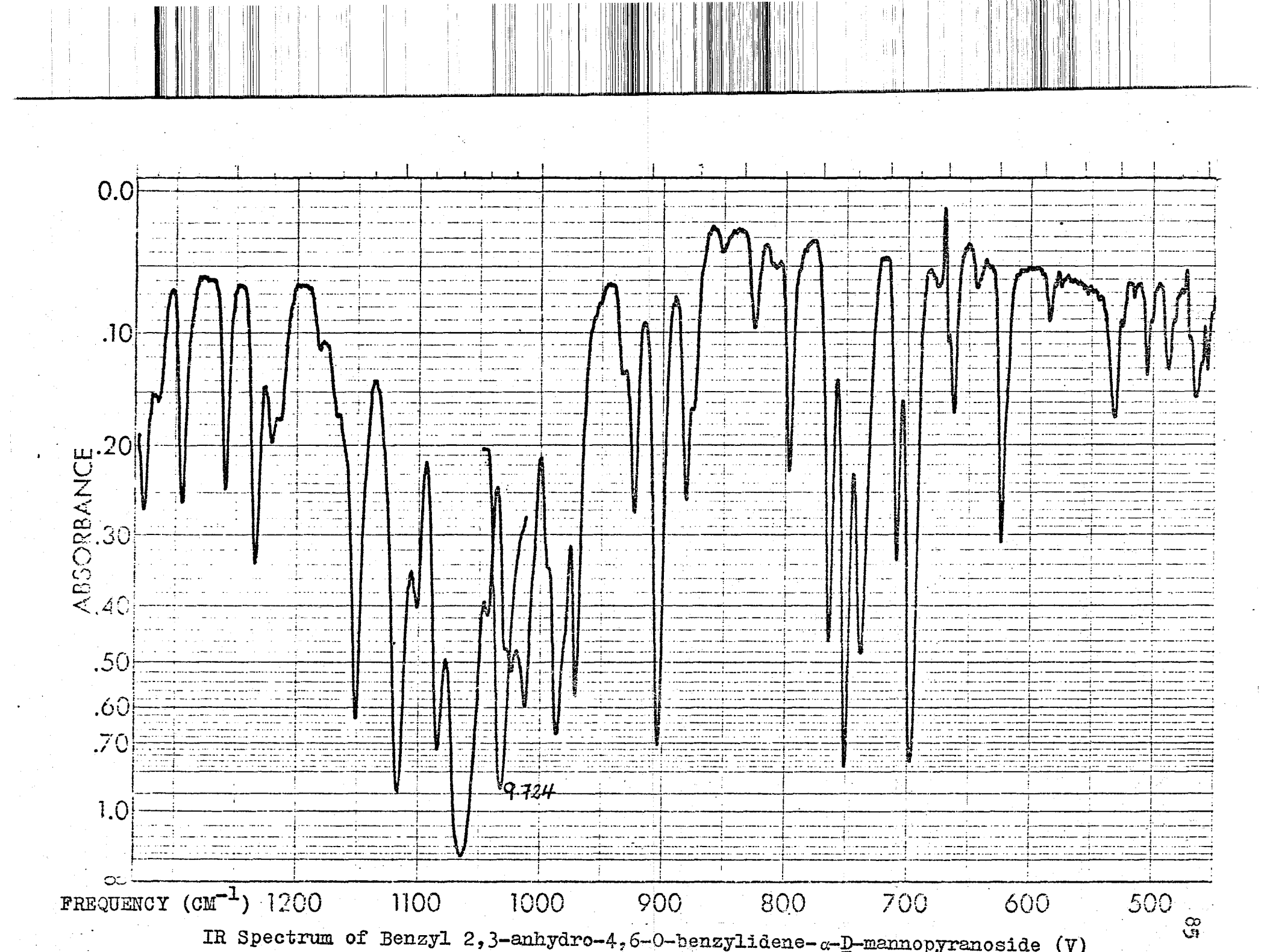

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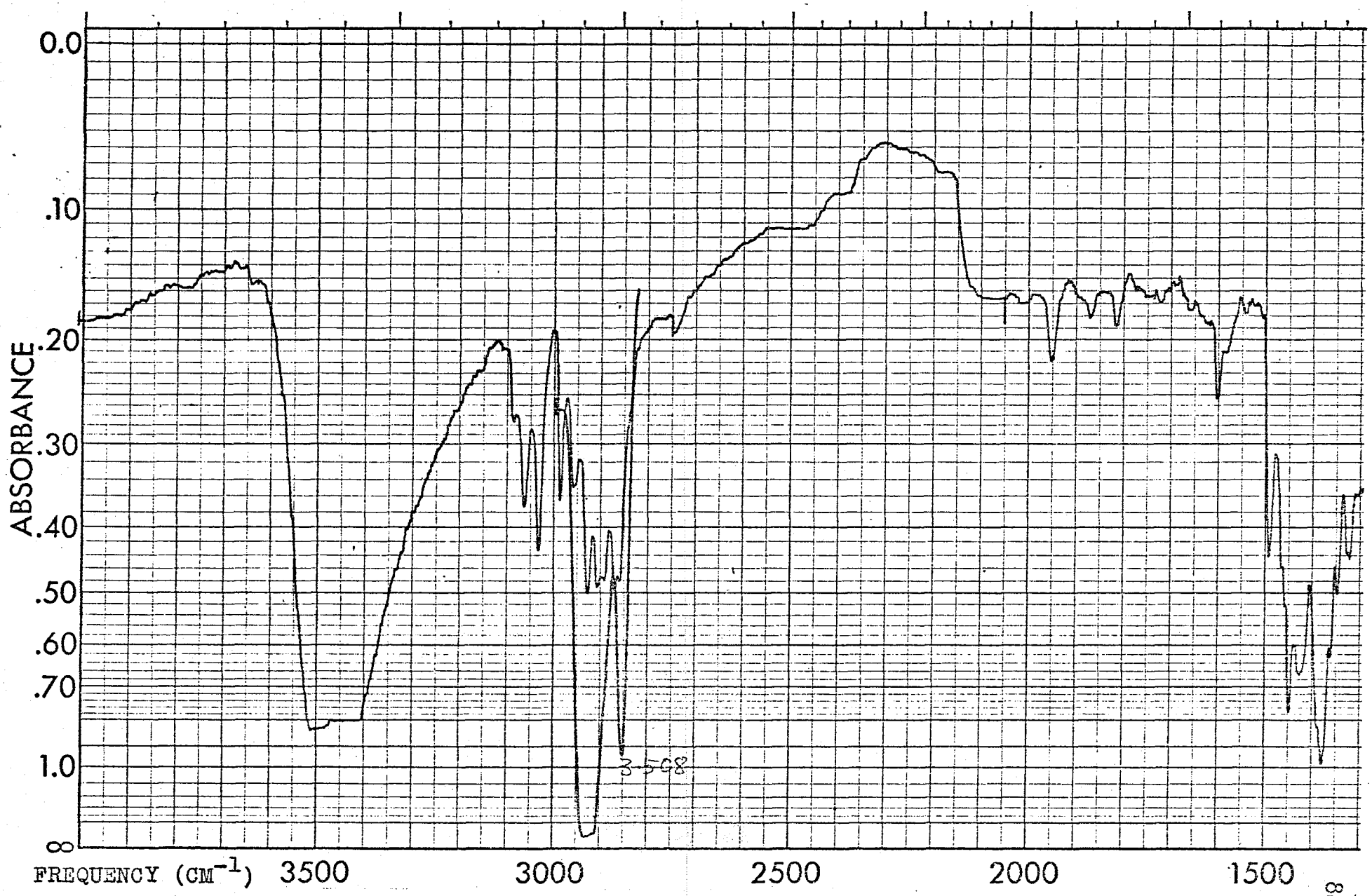
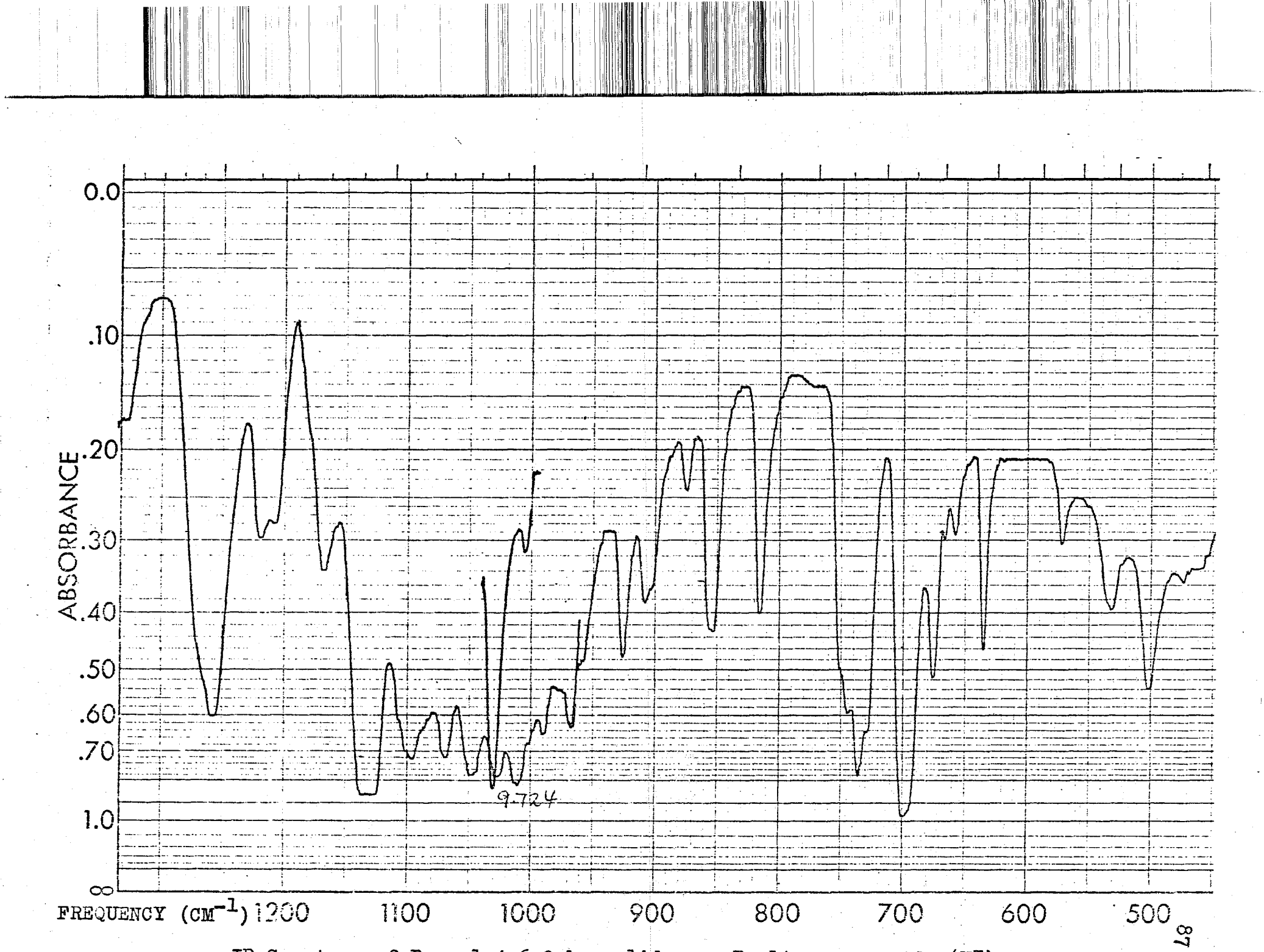
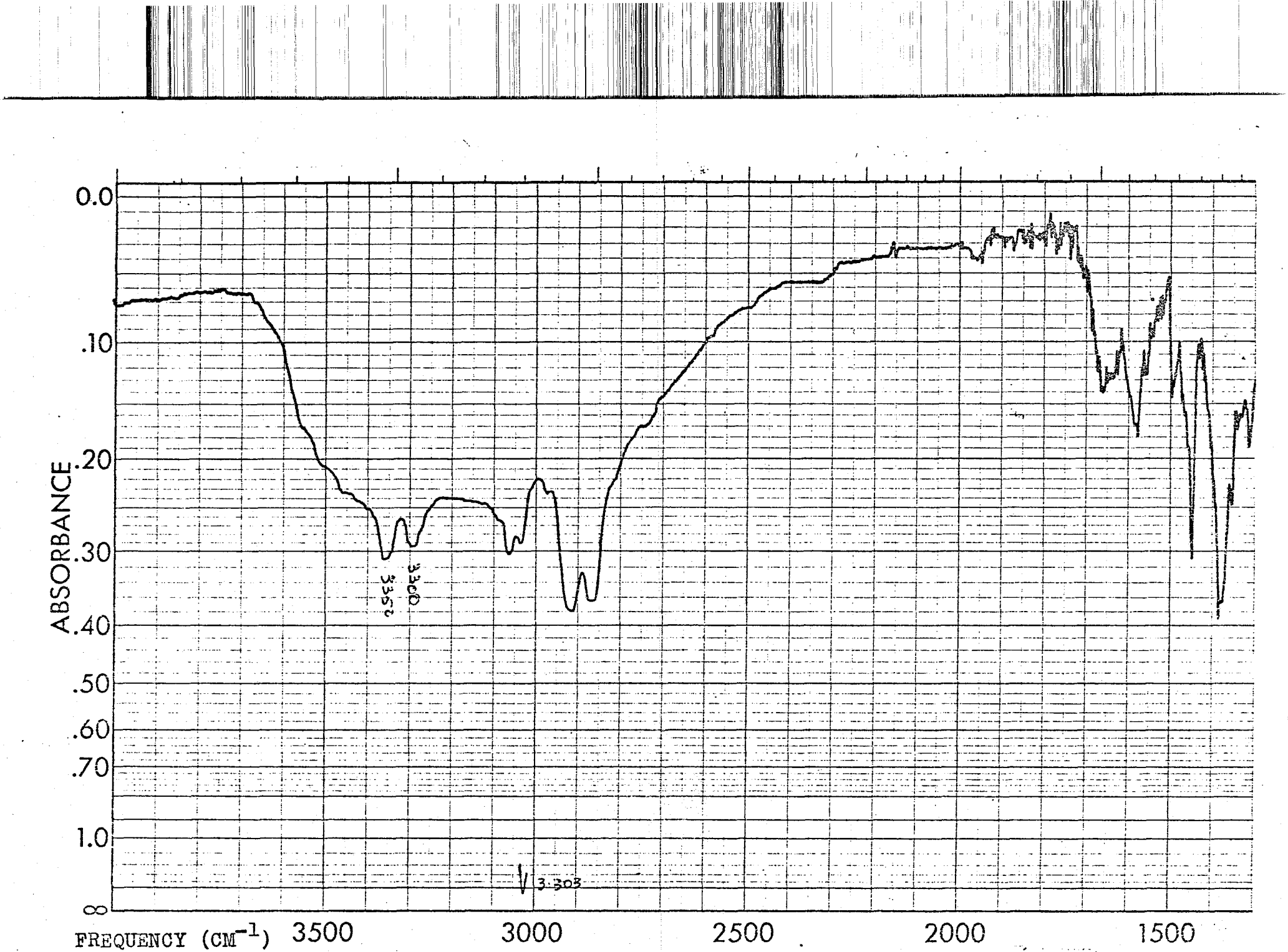


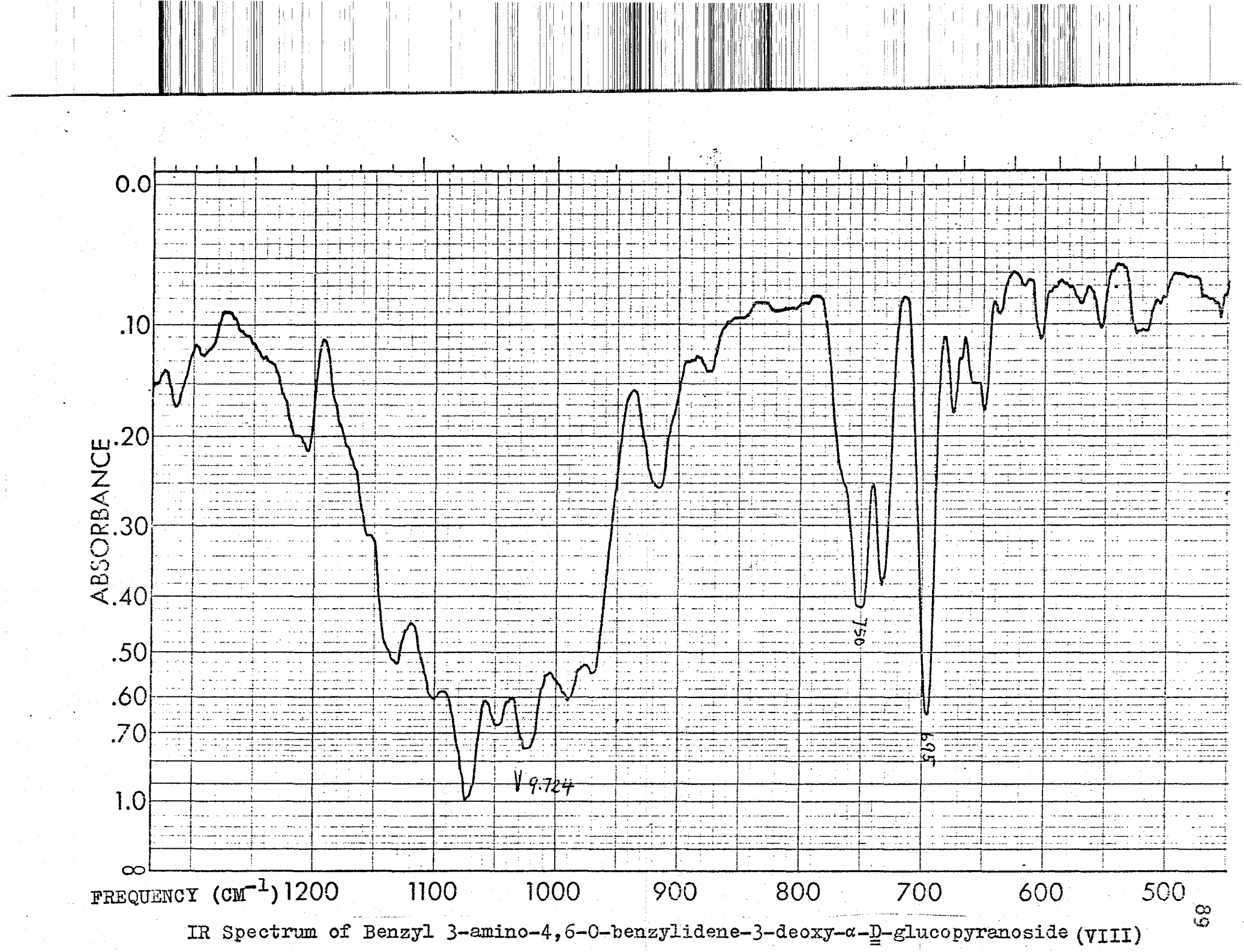
IR Spectrum of Benzyl 4,6-O-benzylidene-α-D-altropyranoside (VI)



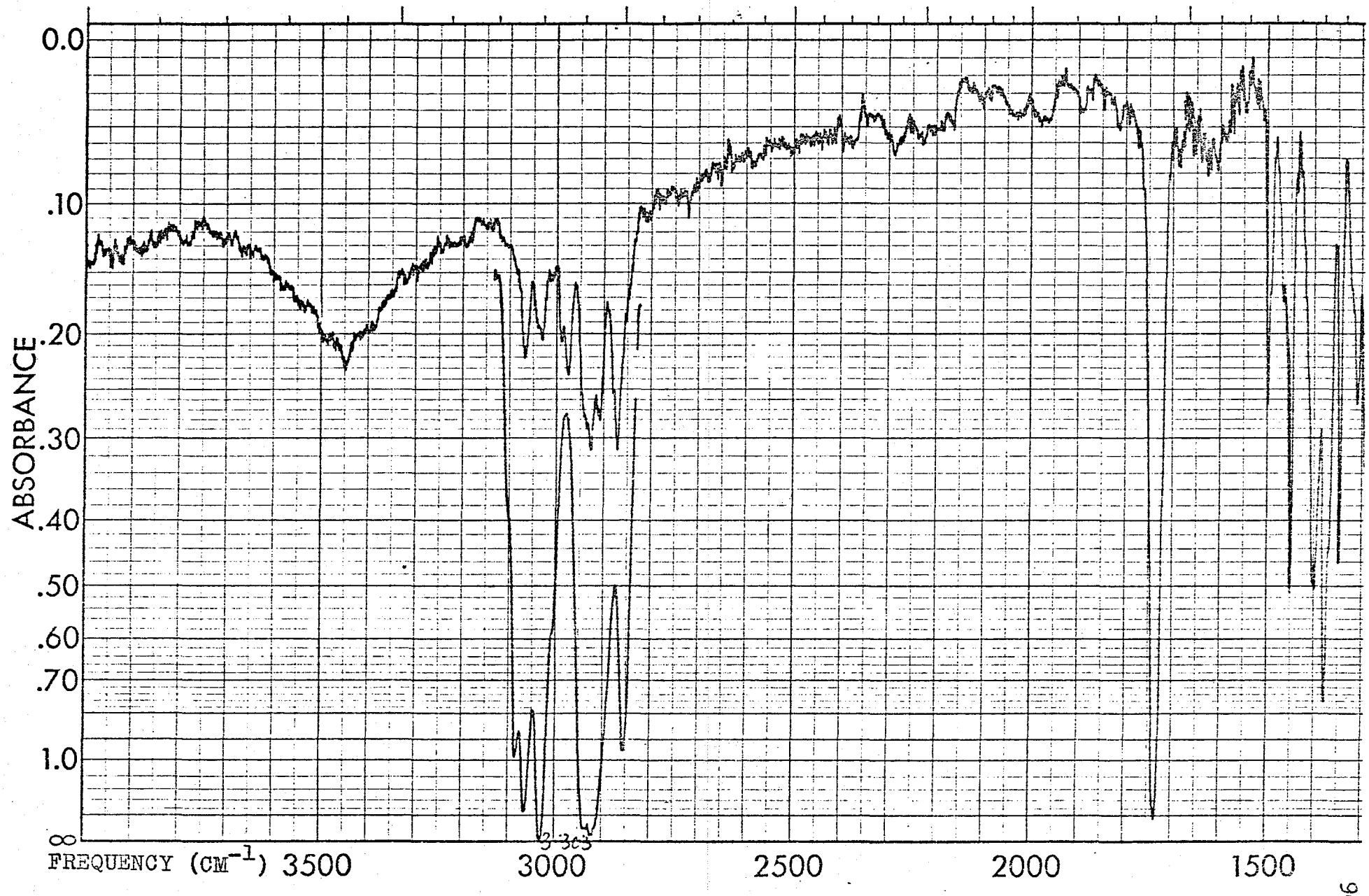
IR Spectrum of Benzyl 4,6-O-benzylidene-α-D-altropyranoside (VI)



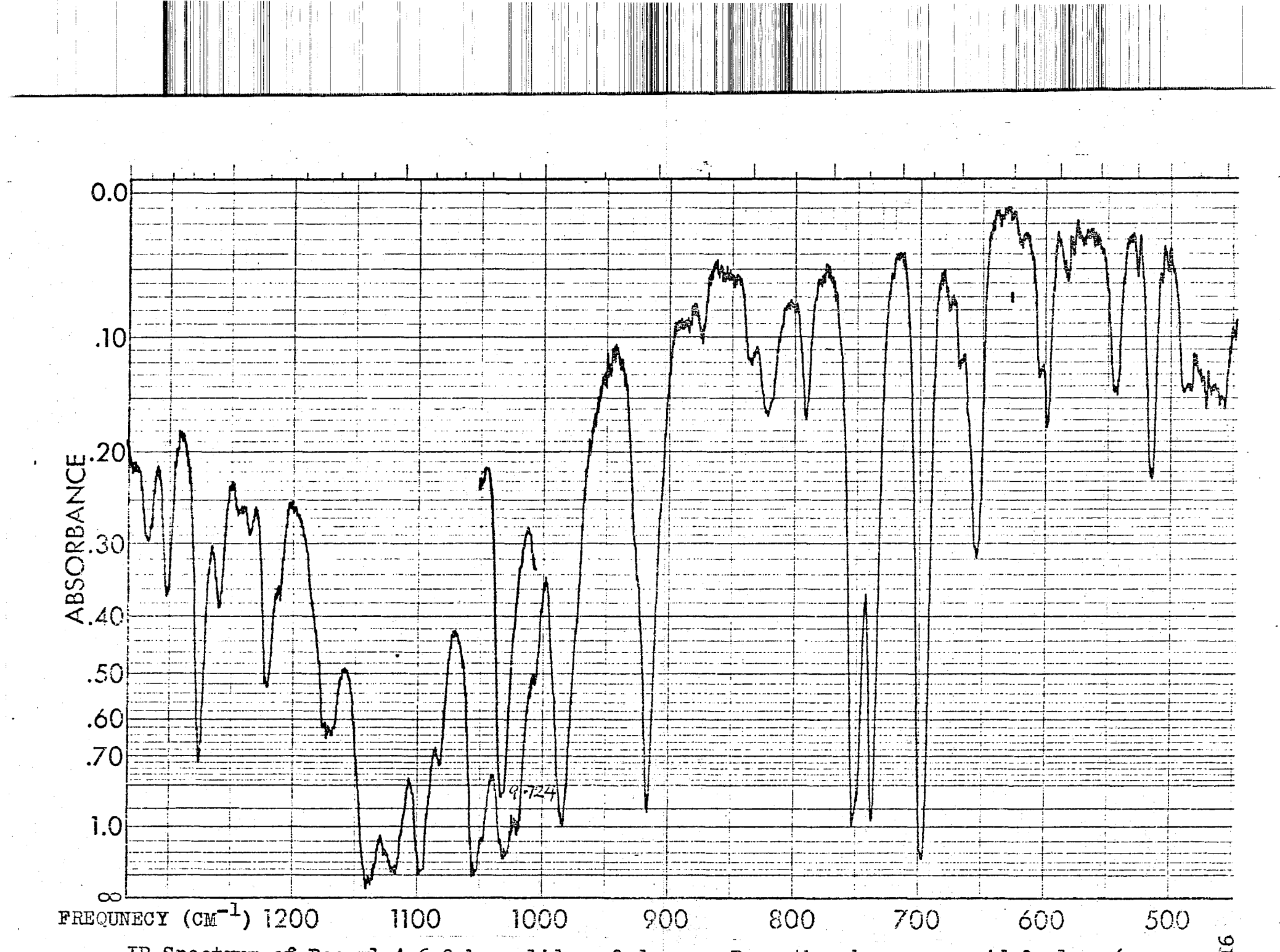
IR Spectrum of Benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII)



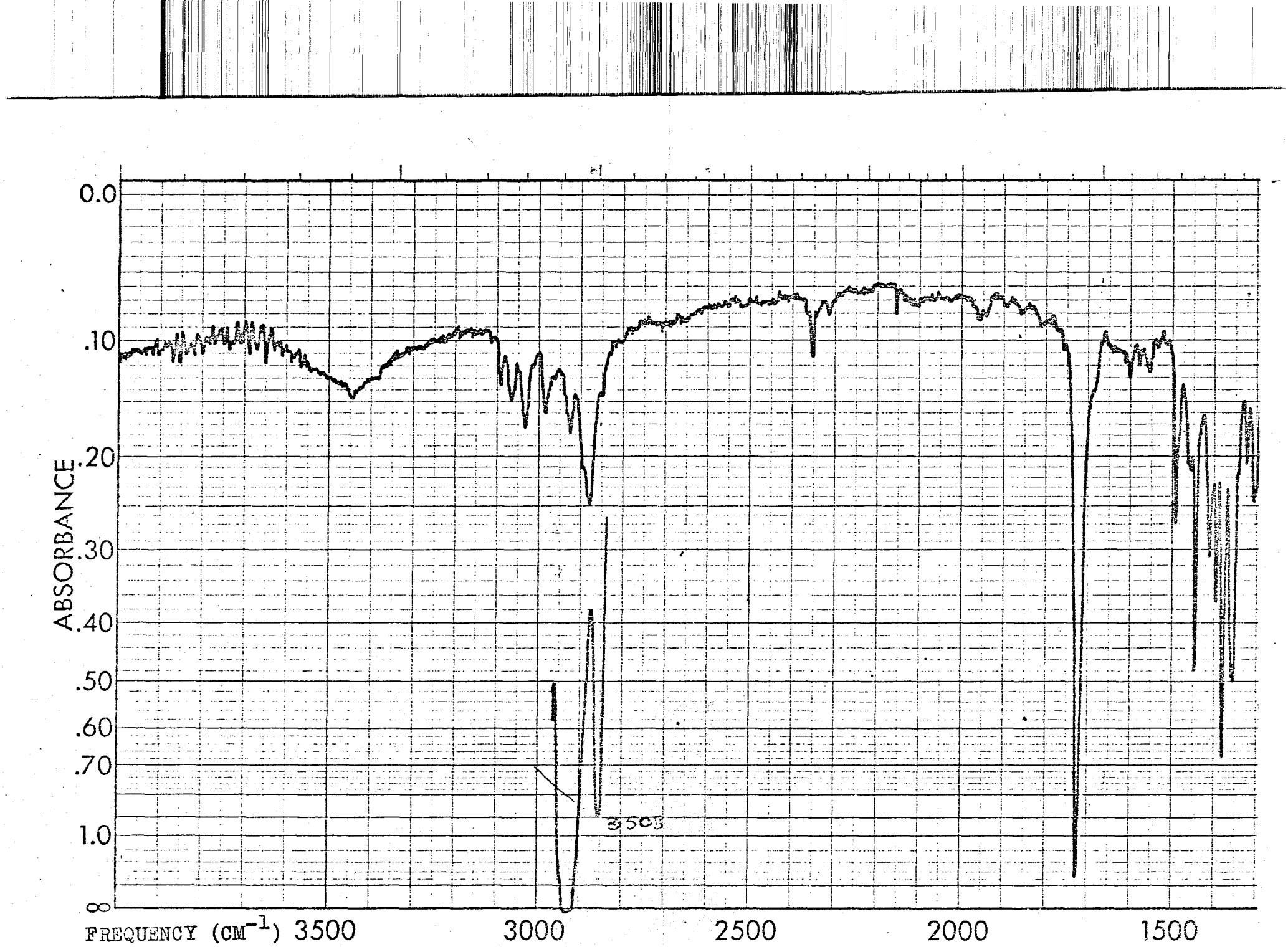
IR Spectrum of Benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII)



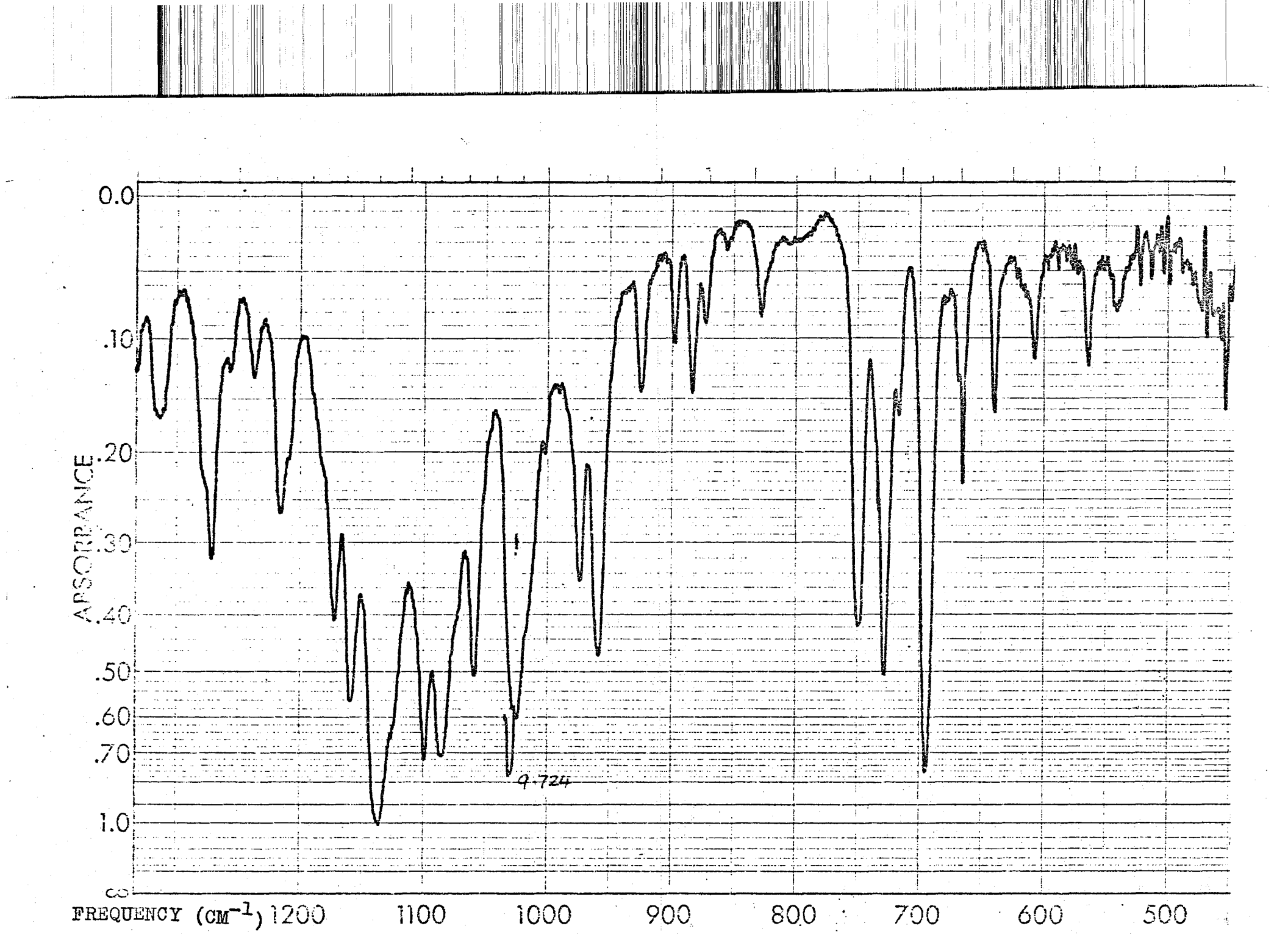
IR Spectrum of Benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XIIIa)



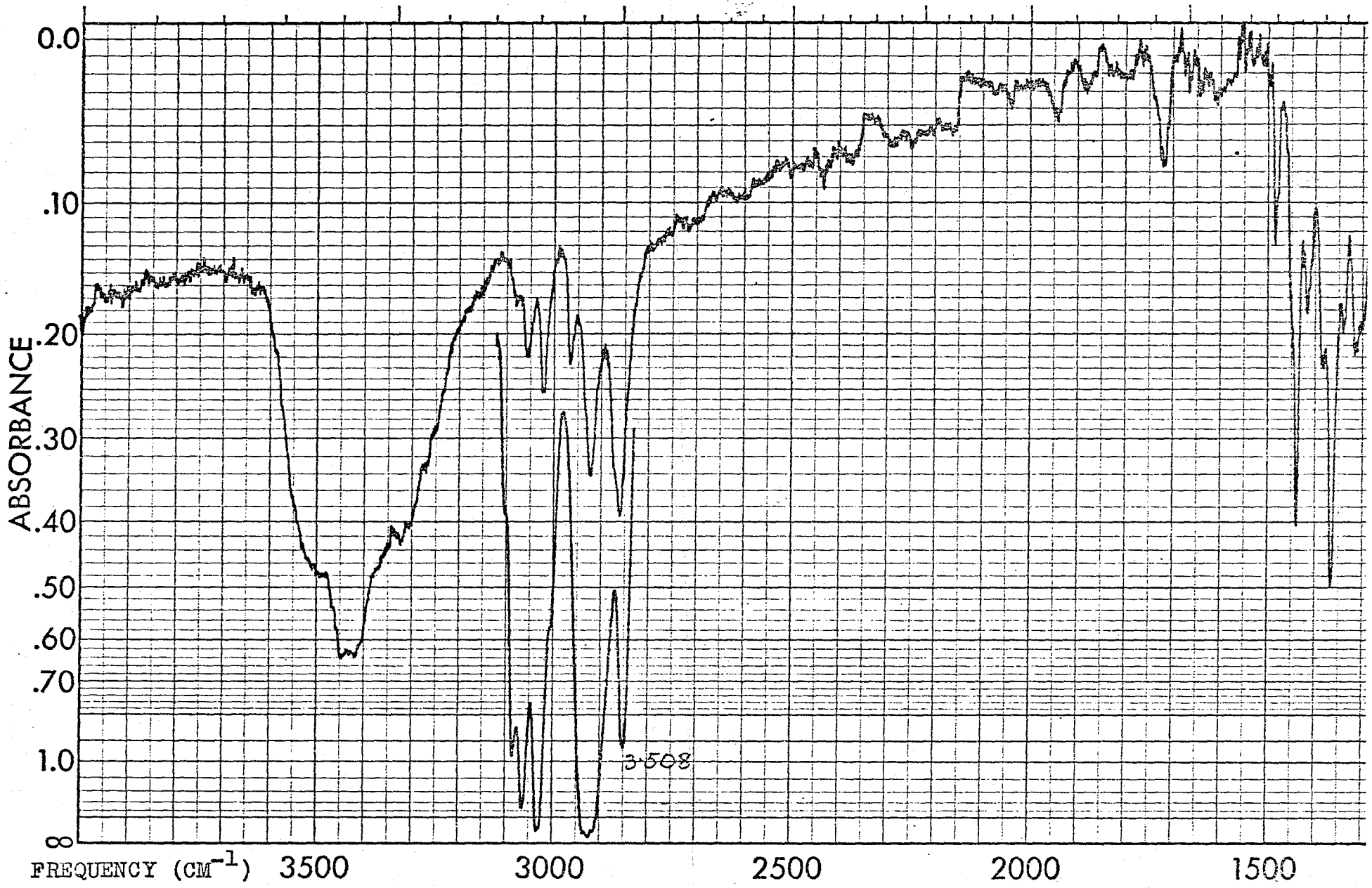
IR Spectrum of Benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XIIIa)



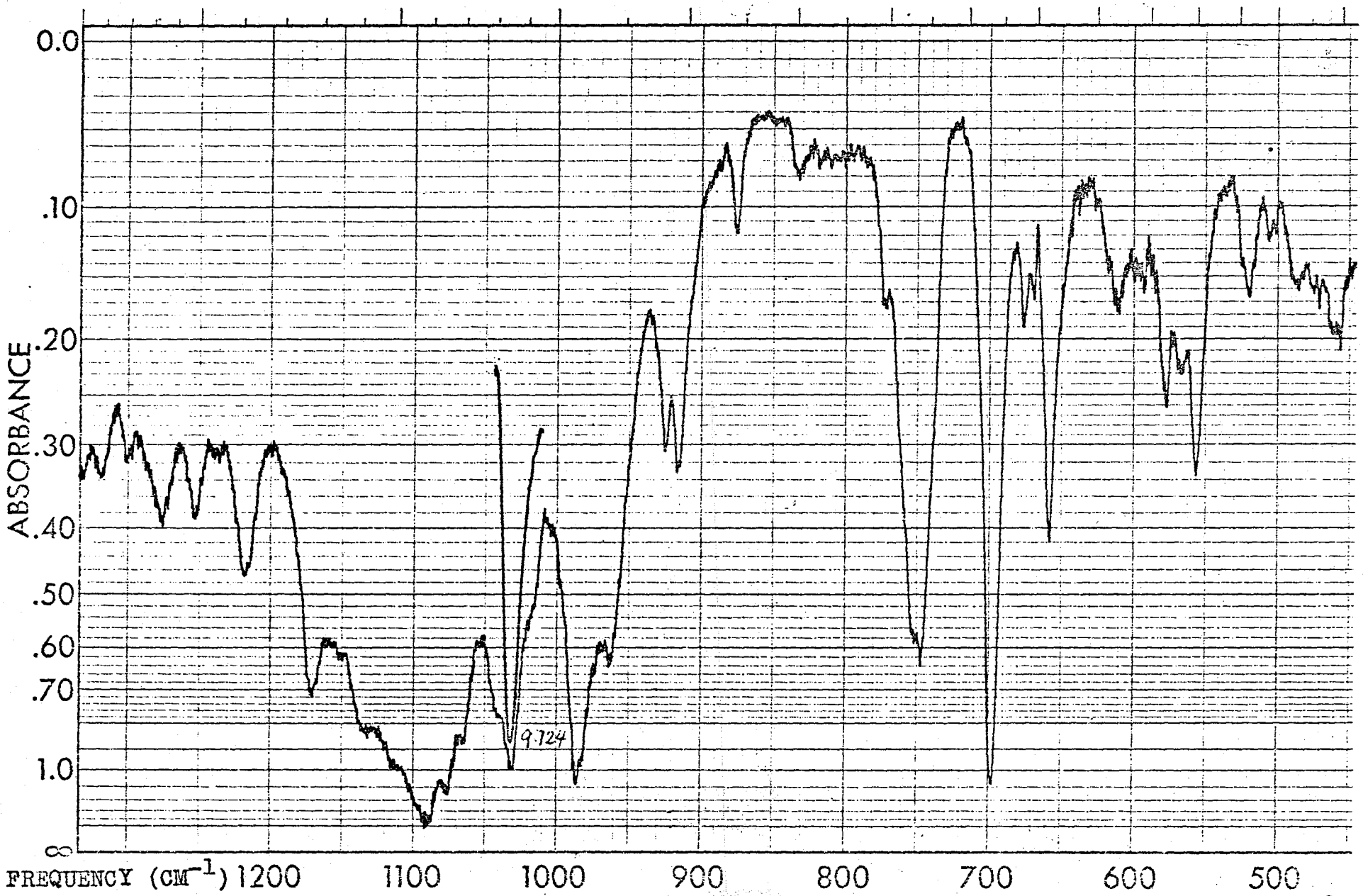
IR Spectrum of Benzyl 4,6-O-benzylidene-2-deoxy-β-D-erythro-hexopyranosid-3-ulose (XIIIb)



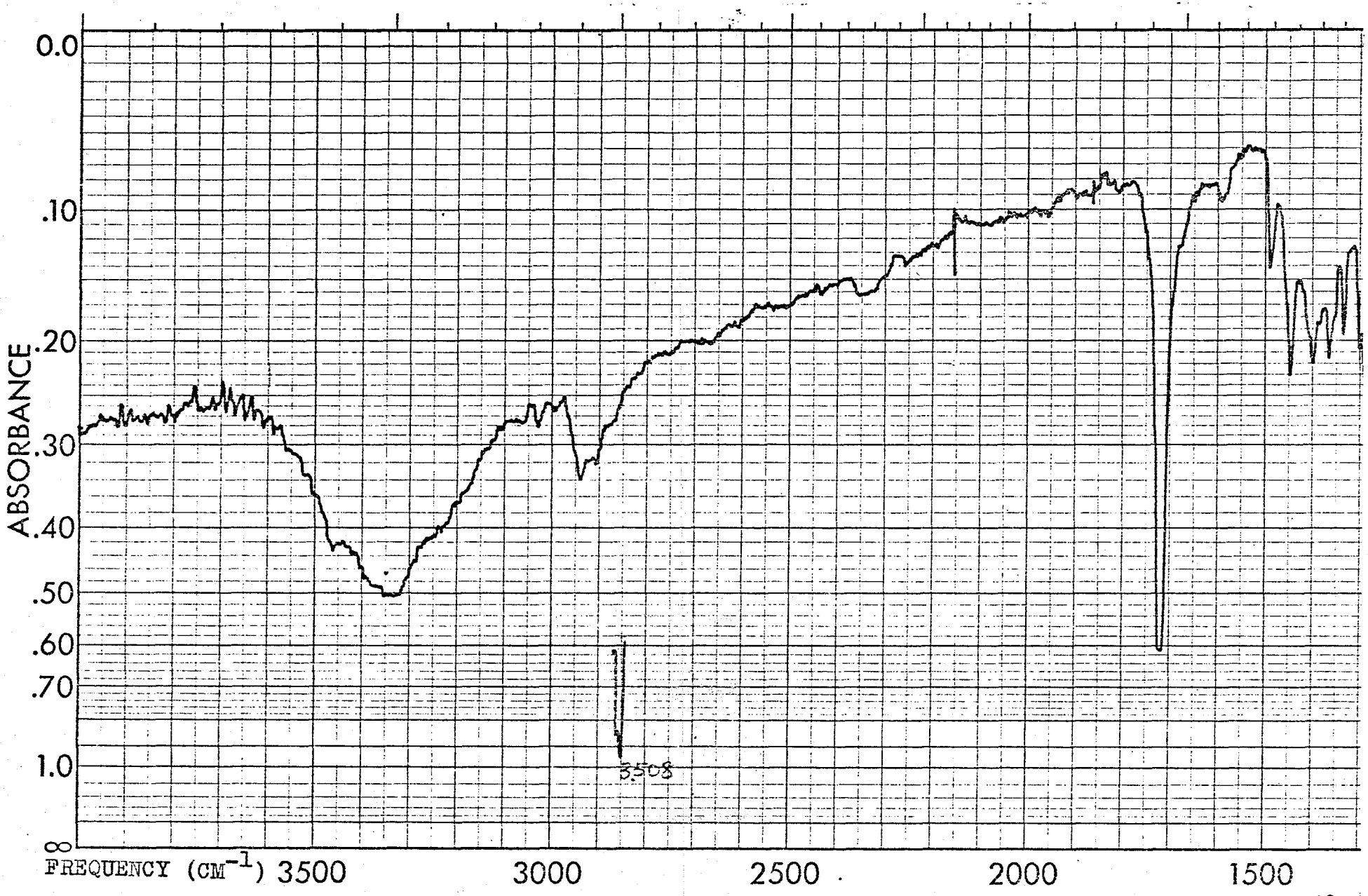
IR Spectrum of Benzyl 4,6-O-benzylidene-2-deoxy-β-D-erythro-hexopyranosid-3-ulose (XIIIb)



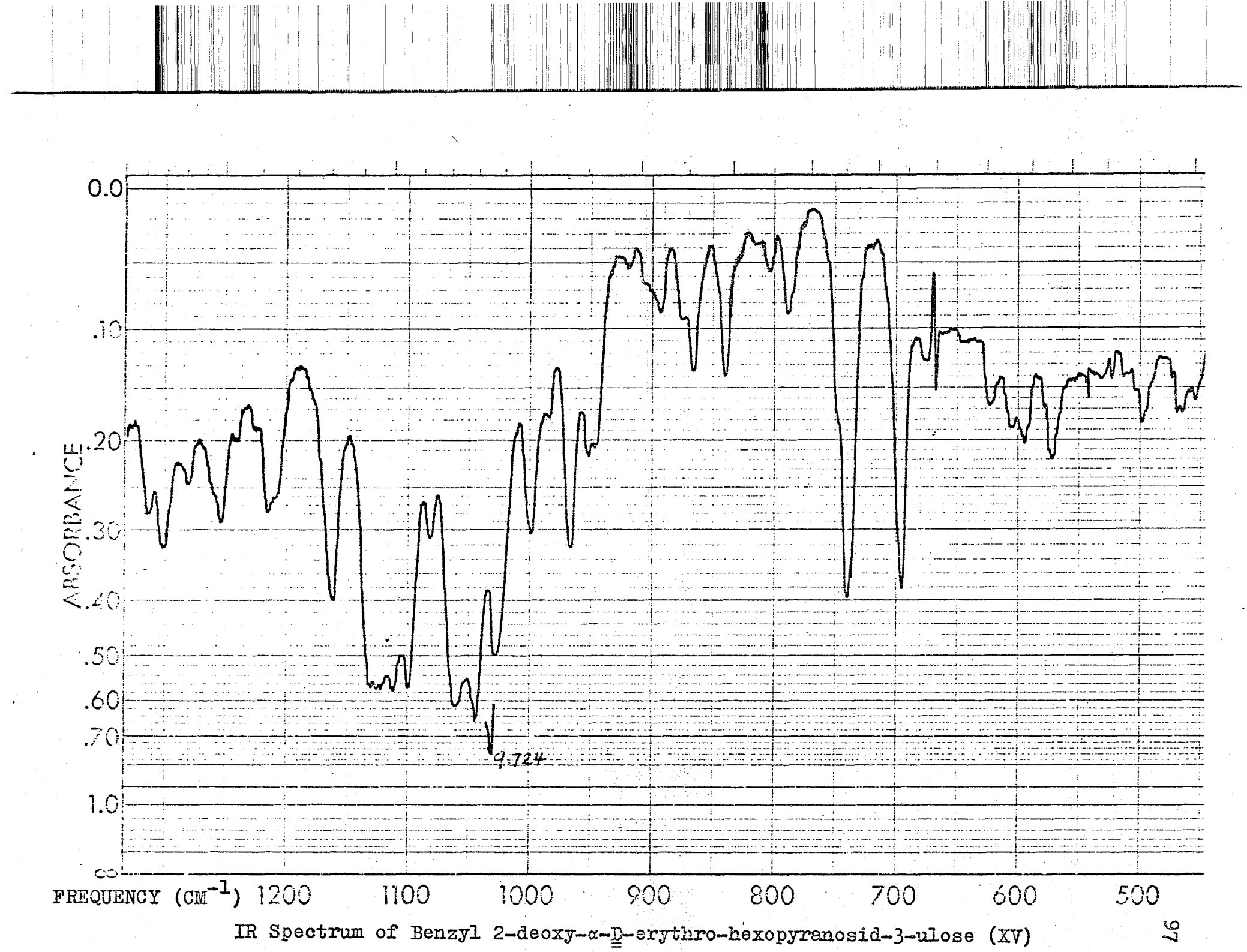
IR Spectrum of 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV)



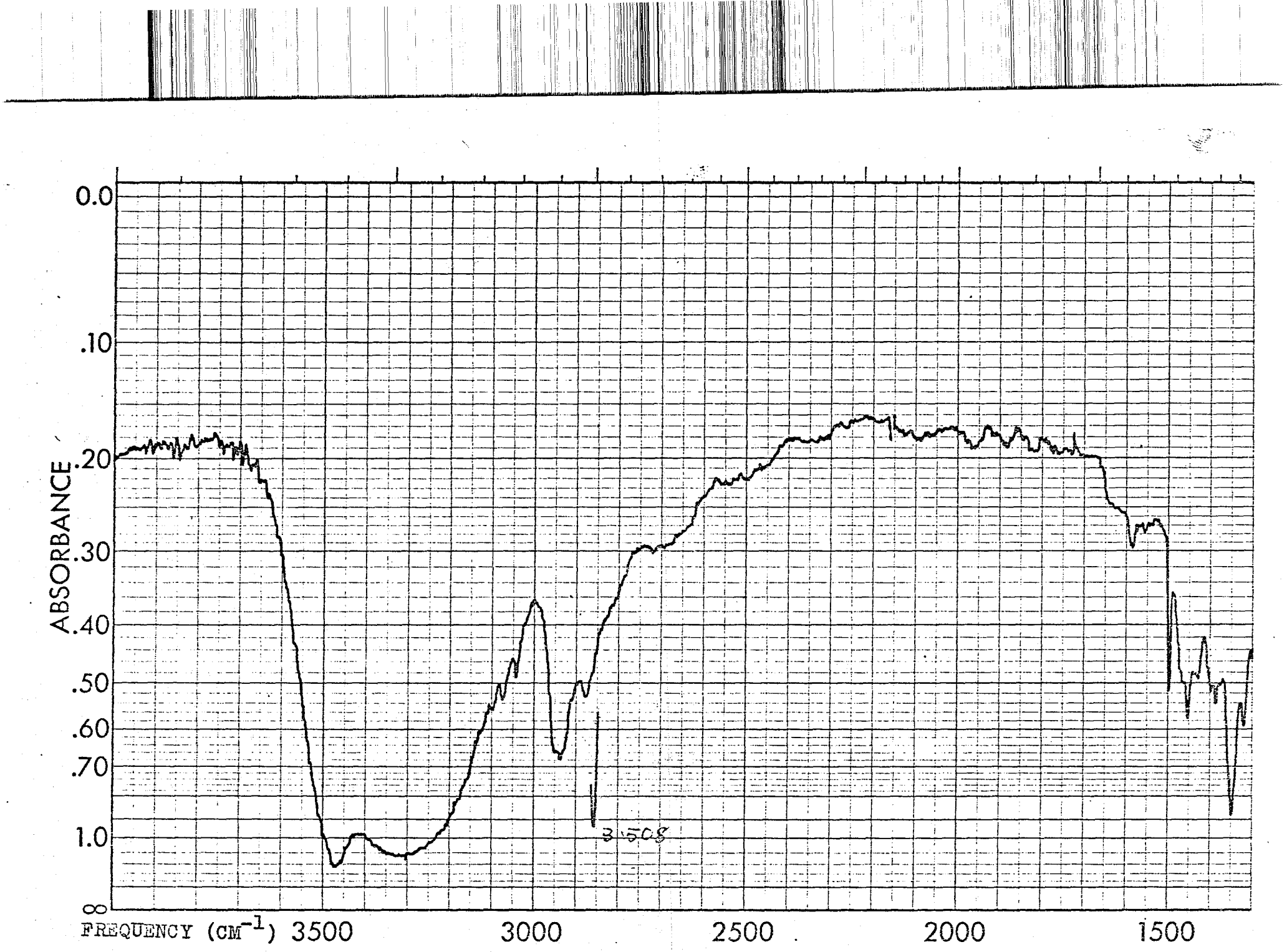
IR Spectrum of 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV)



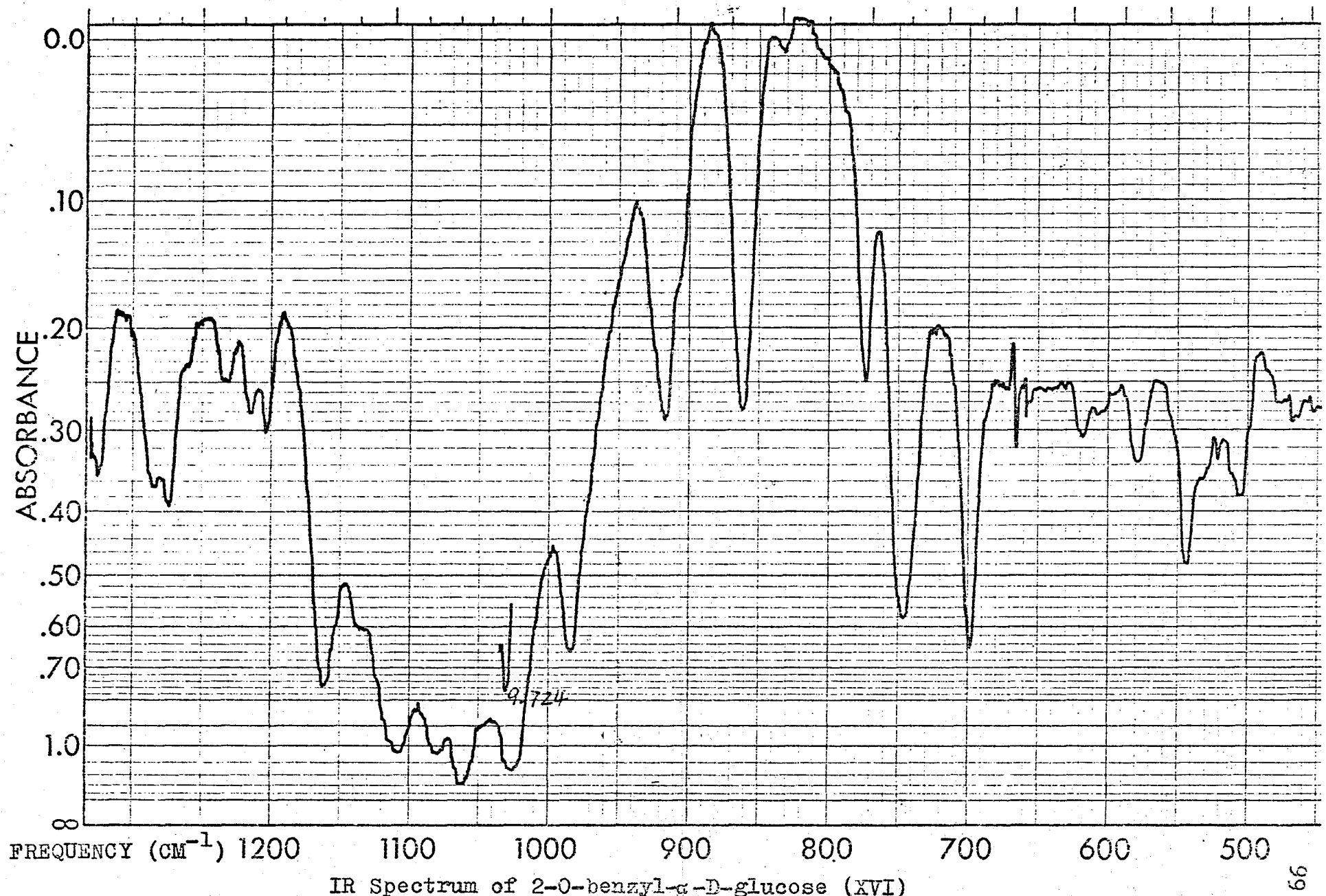
IR Spectrum of Benzyl 2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XV)



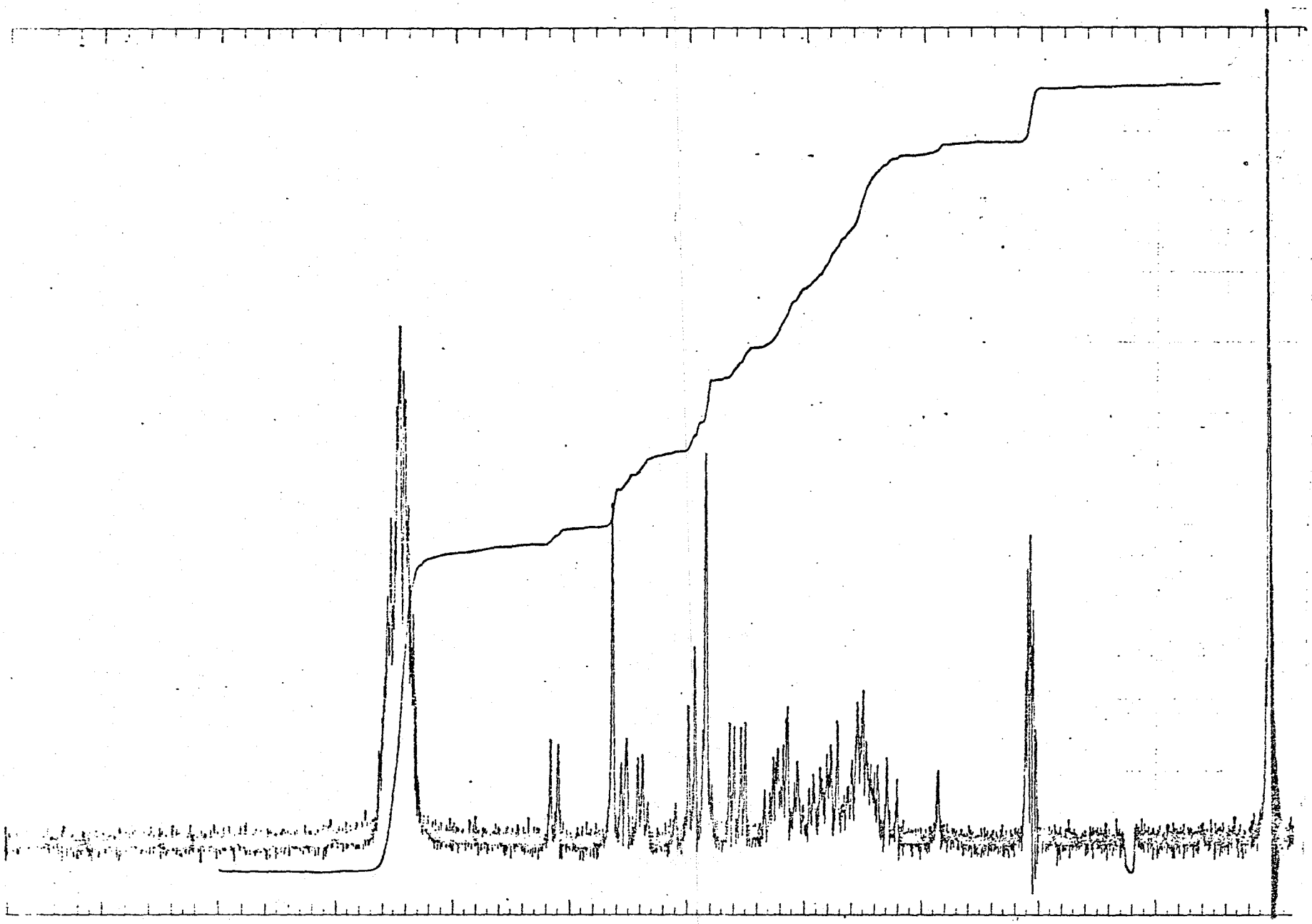
IR Spectrum of Benzyl 2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XV)



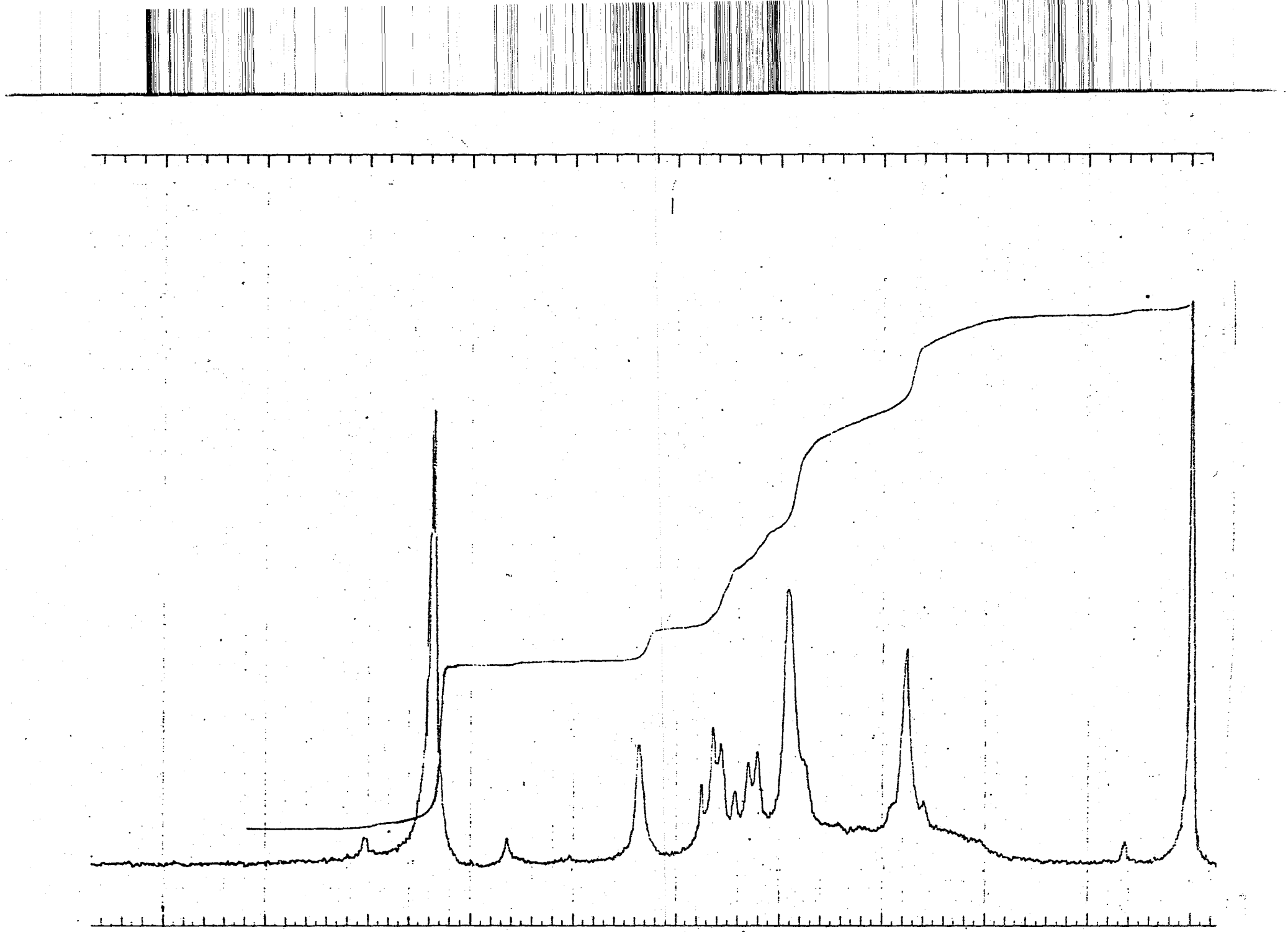
IR Spectrum of 2-O-benzyl-α-D-glucose (XVI)



IR Spectrum of 2-O-benzyl-α-D-glucose (XVI)



NMR Spectrum of 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV)



NMR Spectrum of Benzyl 2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XV)

Plate No. 1

Solvent System: Carbon Tetrachloride:Chloroform (1:1)

Samples:
1. Benzyl 4,6-O-benzylidene-2,3-di-O-methanesulfonyl-α-D-glucopyranoside (III)
2. Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV)
3. Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside (V)

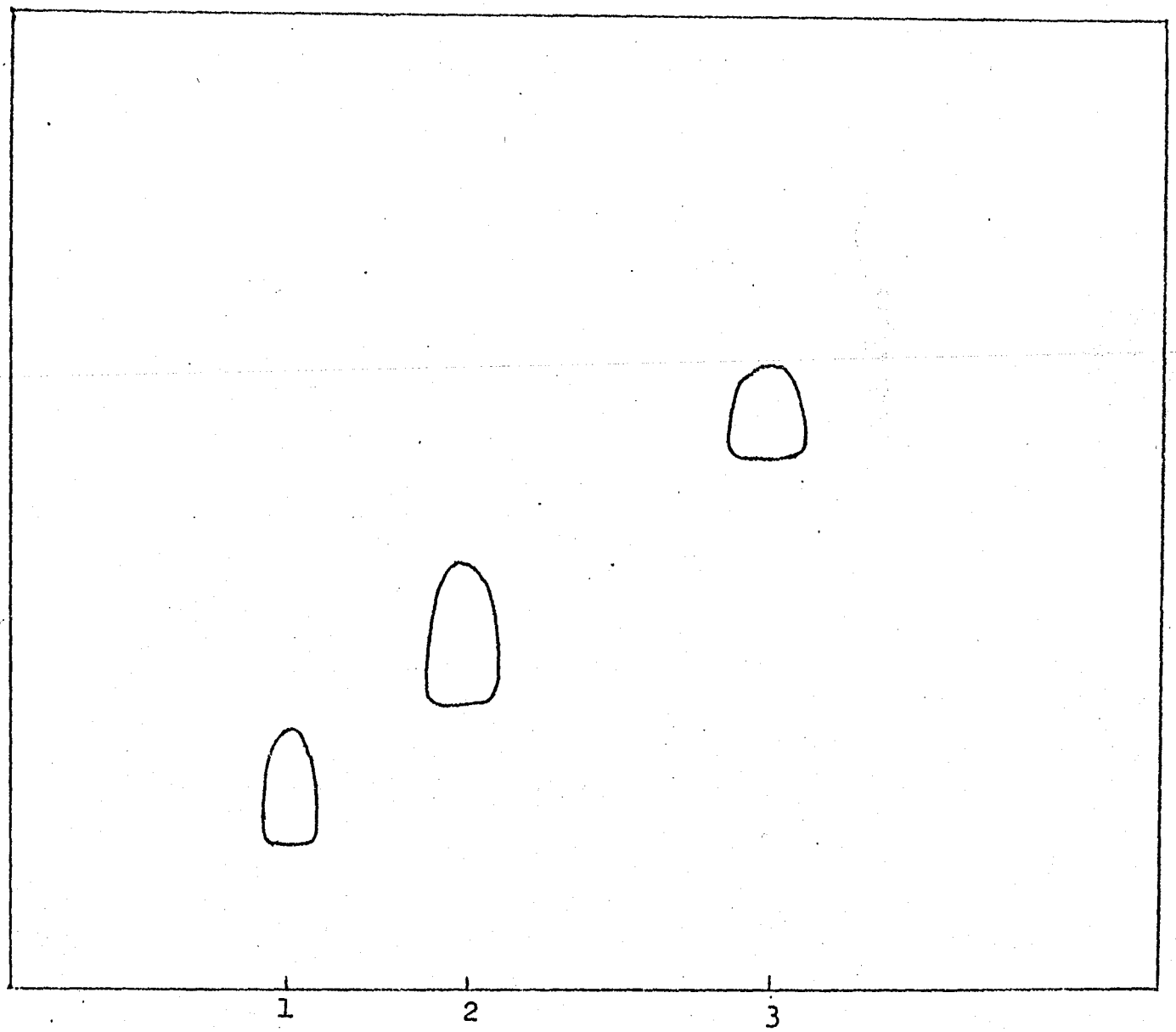


Plate No. 2

Solvent System: Carbon tetrachloride:Chloroform (1:1)

Samples:
1. Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV)
2. Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside (V)
3. Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII)
4. Benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII)

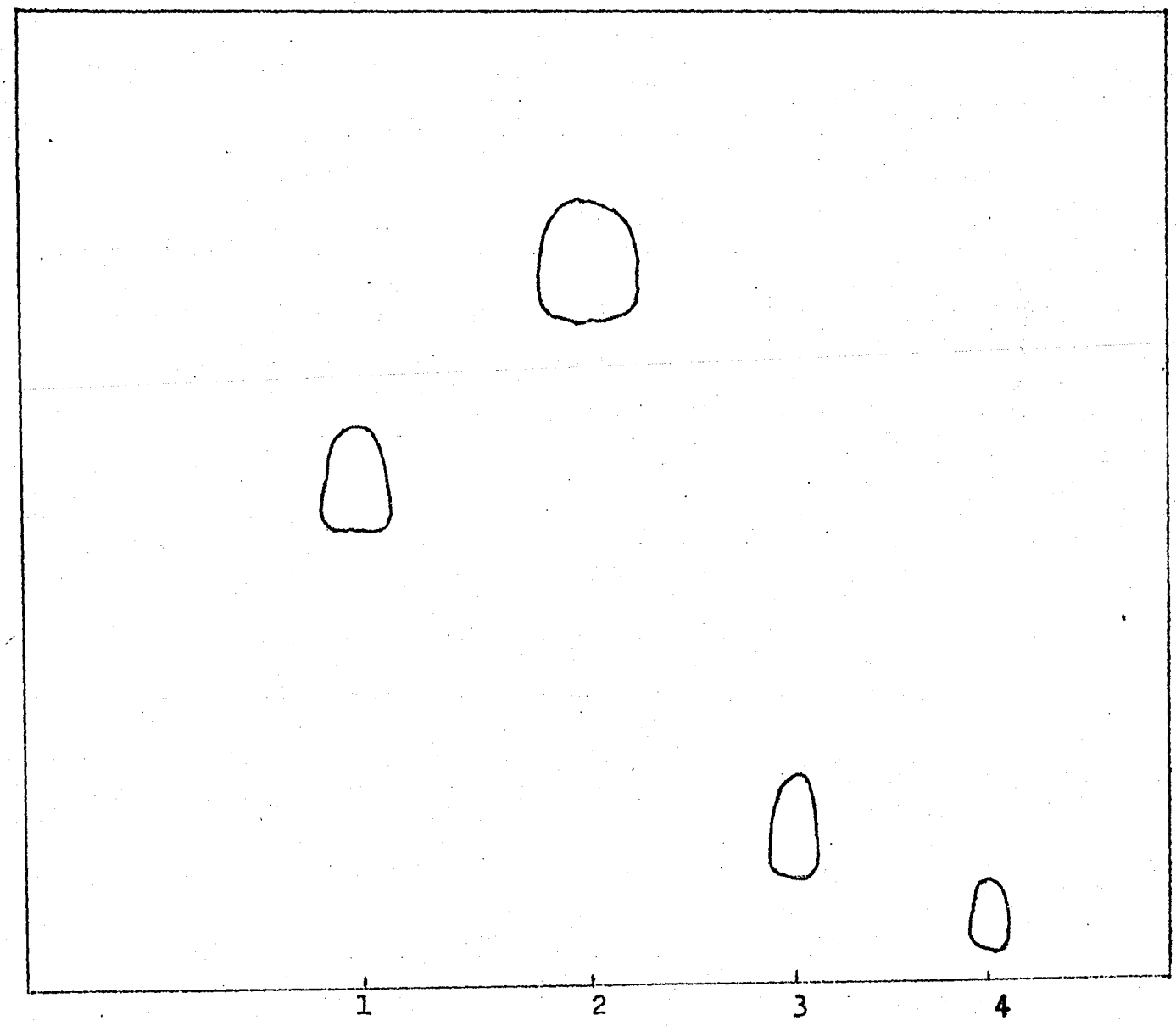


Plate No. 3

Solvent System: Chloroform:Benzene:Acetone (65:30:5)

Samples:
1. Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (VII)
2. Deamination product of VII with nitrous acid
3. Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV)

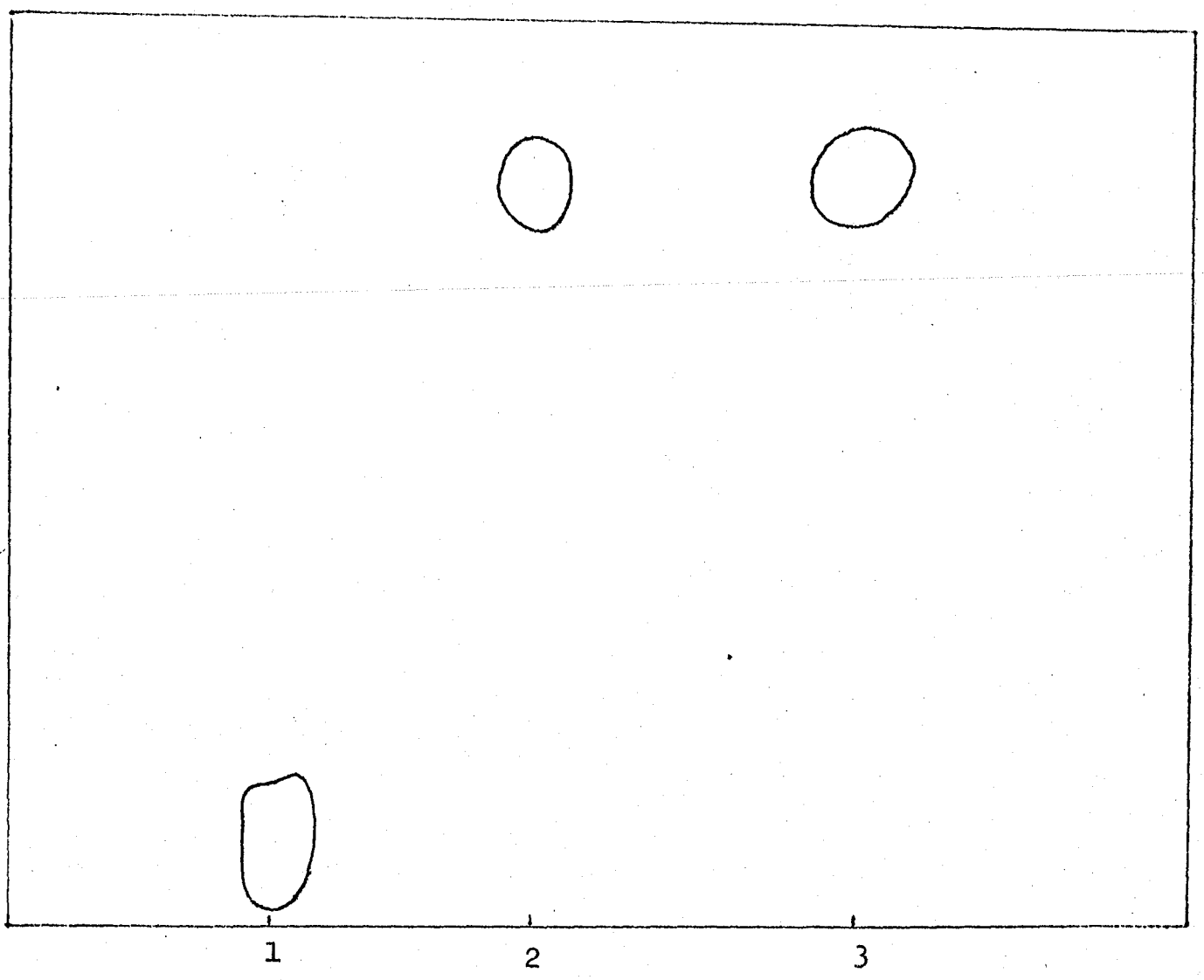


Plate No. 4

Solvent System: Chloroform:Benzene:Methanol (85:25:10)

Samples:
1. Benzyl 3-amino-4,6-O-benzylidene-3-deoxy-α-D-glucopyranoside (VIII)
2. Deamination products of VIII with nitrous acid
3. Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (IV)

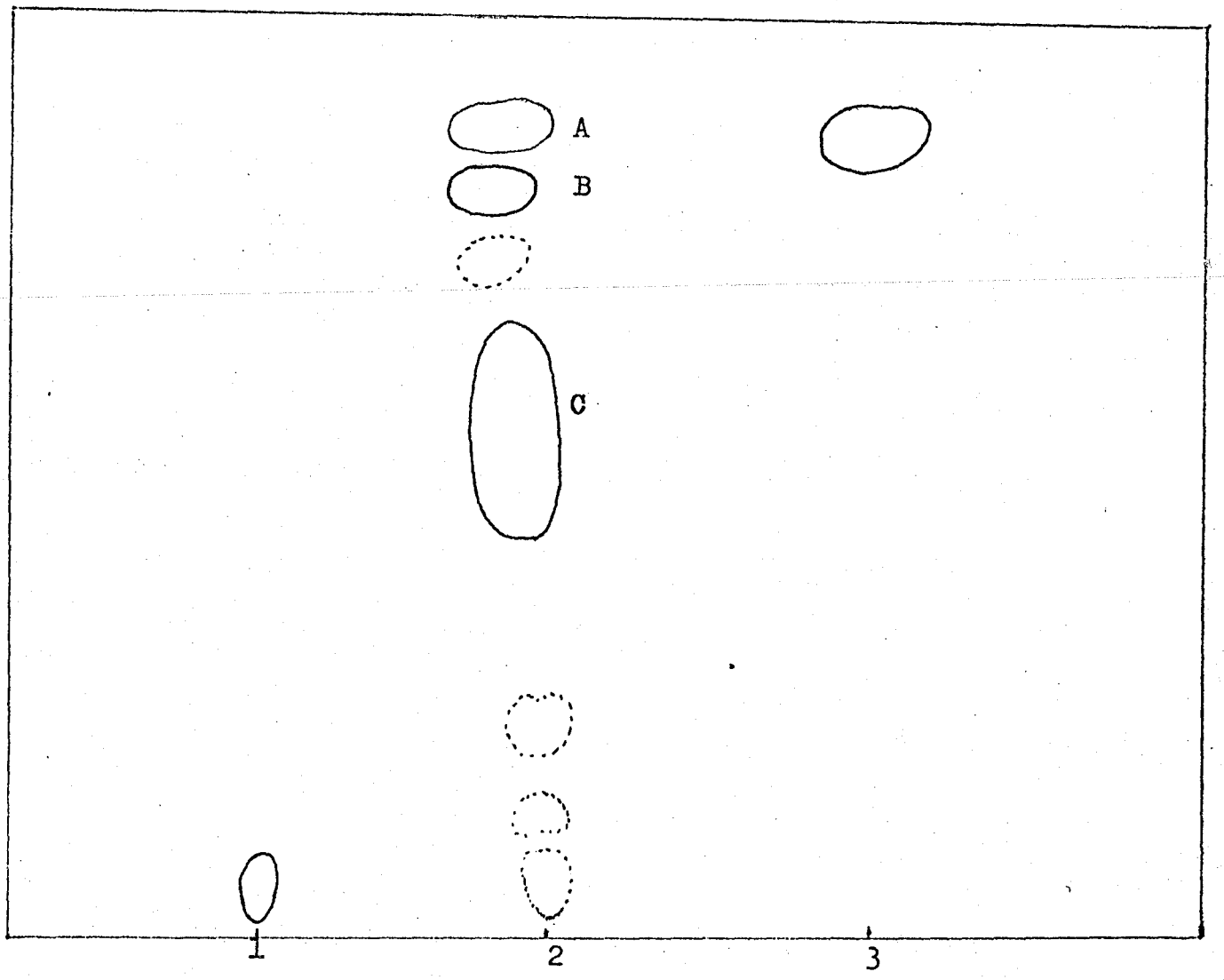


Plate No. 5

Solvent System: Chloroform:Benzene:Acetone (65:30:5)

Samples:
1. Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-mannopyranoside (XII)
2. Deamination products of XII with nitrous acid
3. Benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XIIIa)
4. 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV)

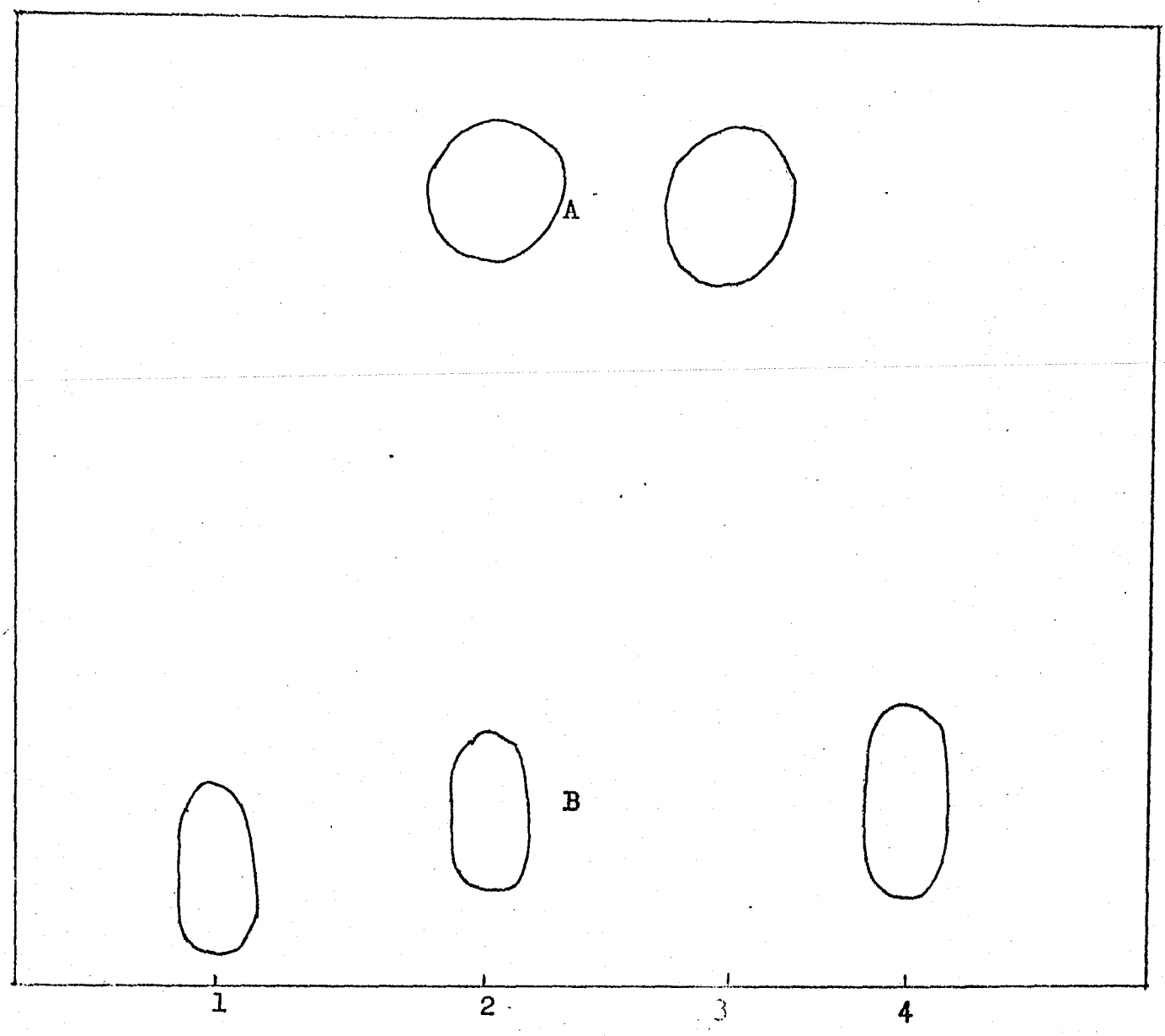


Plate No. 6

Solvent System: Chloroform:Benzene:Acetone (65:30:5)

Samples:
1. Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (XXIIa)
2. Deamination of XXIIa with nitrous acid
3. 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV)
4. Benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XIIIa)

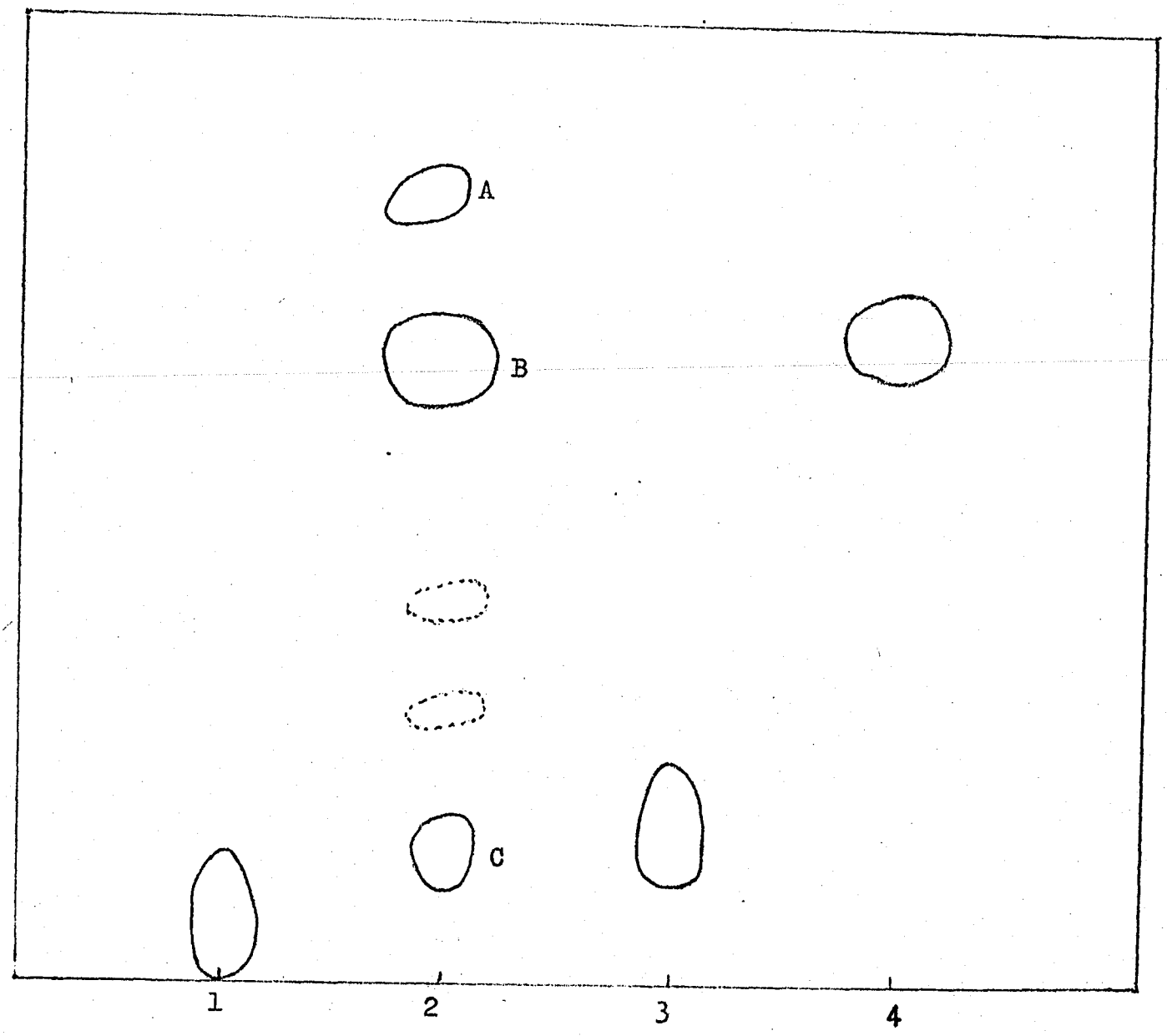


Plate No. 7

Solvent System: Chloroform:Benzene:Acetone (65:30:5)

Samples:
1. Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (XXIIb)
2. Deamination products of XXIIb with nitrous acid
3. Benzyl 4,6-O-benzylidene-2-deoxy-β-D-erythro-hexopyranosid-3-ulose (XIIIb)
4. Benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XIIIa)

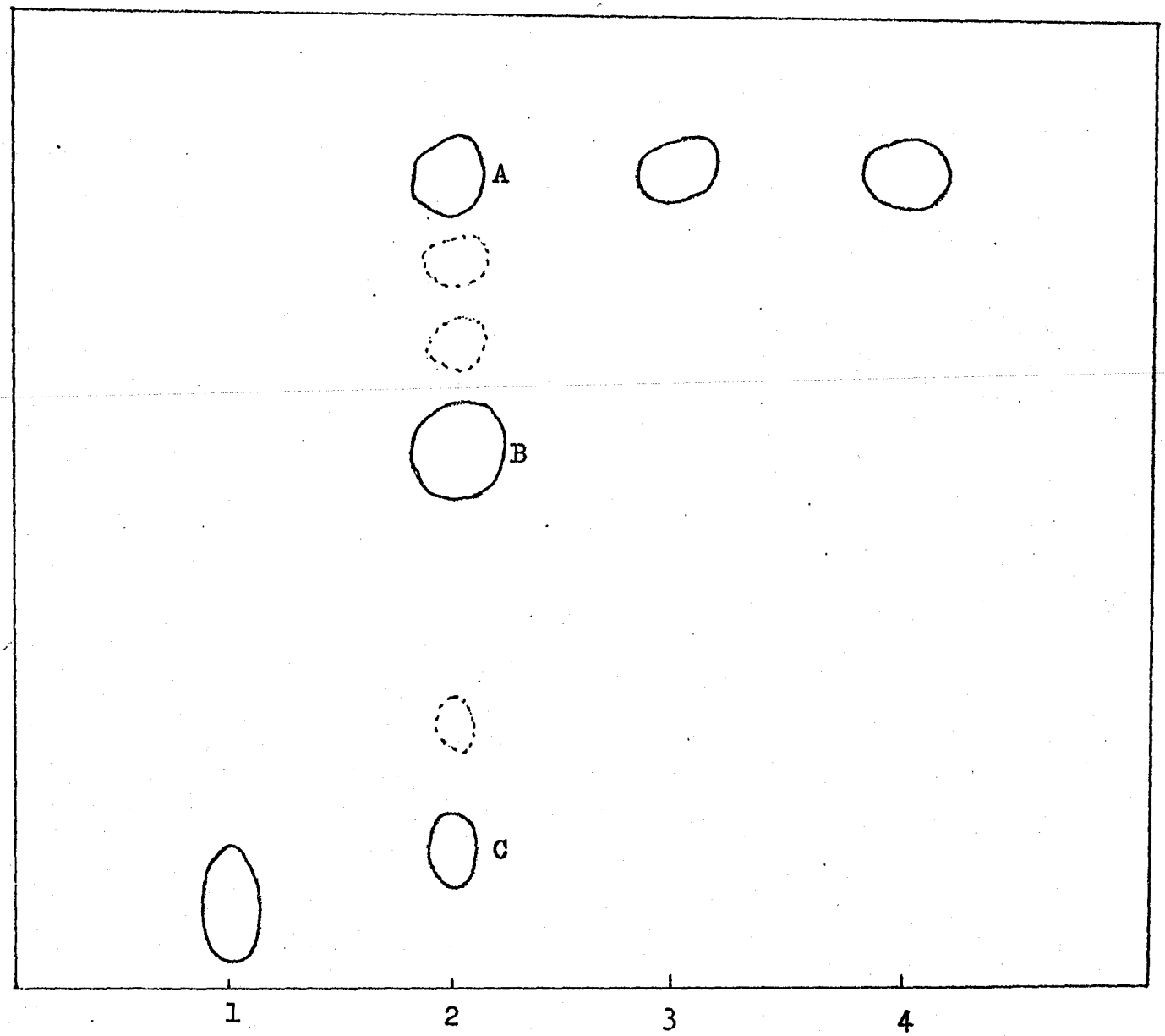


Plate No. 8

Solvent System: Chloroform:Methanol (95:5)

Samples:
1. Benzyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XIIIa)
2. Benzyl 2-deoxy-α-D-erythro-hexopyranosid-3-ulose (XV)
3. 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV)

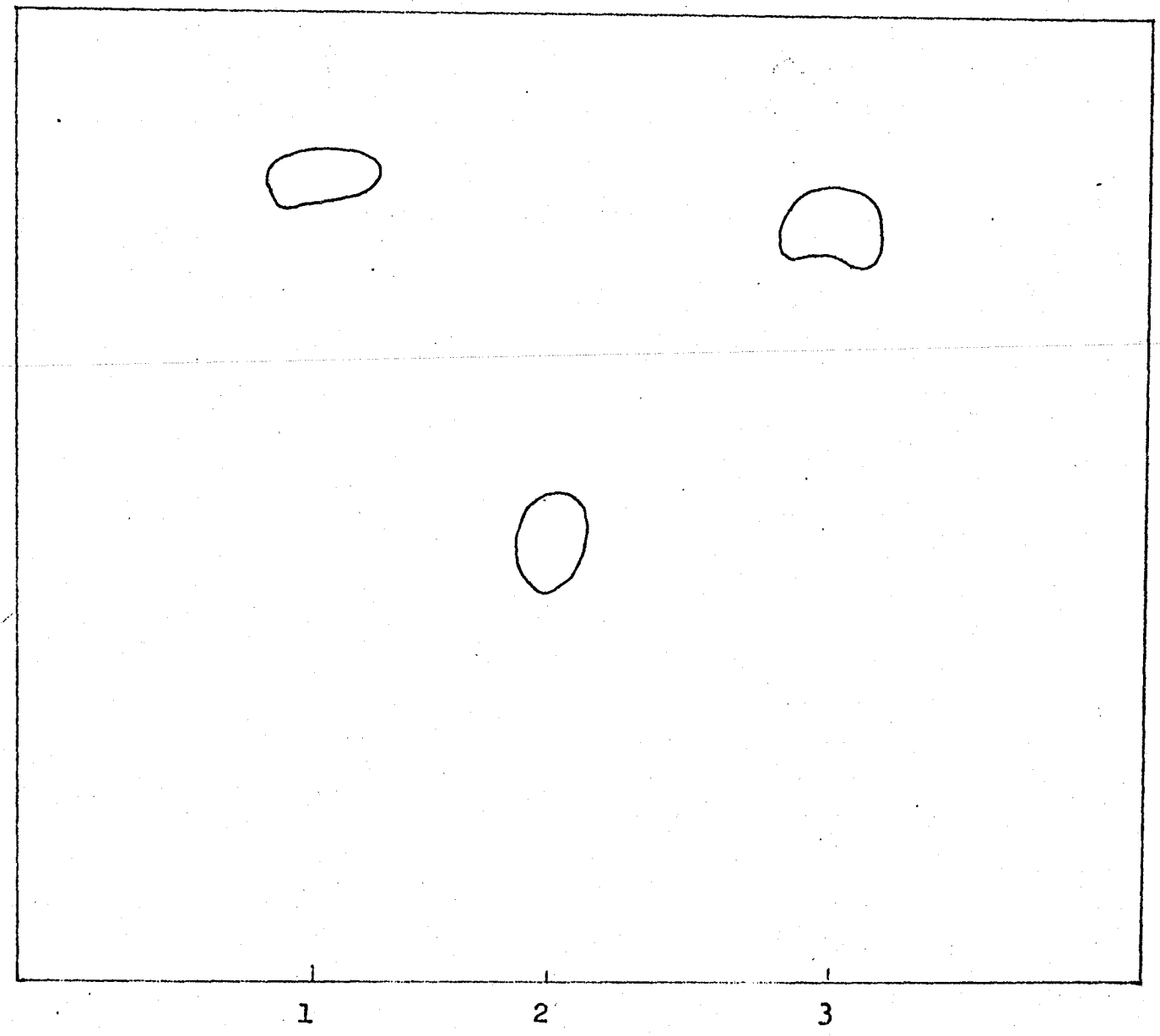


Plate No. 9

Solvent System: Chloroform:Methanol:Benzene(88:8:4)

Samples: 1. 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranose (XIV)
2. 2-O-benzyl-α-D-glucopyranose (XVI)

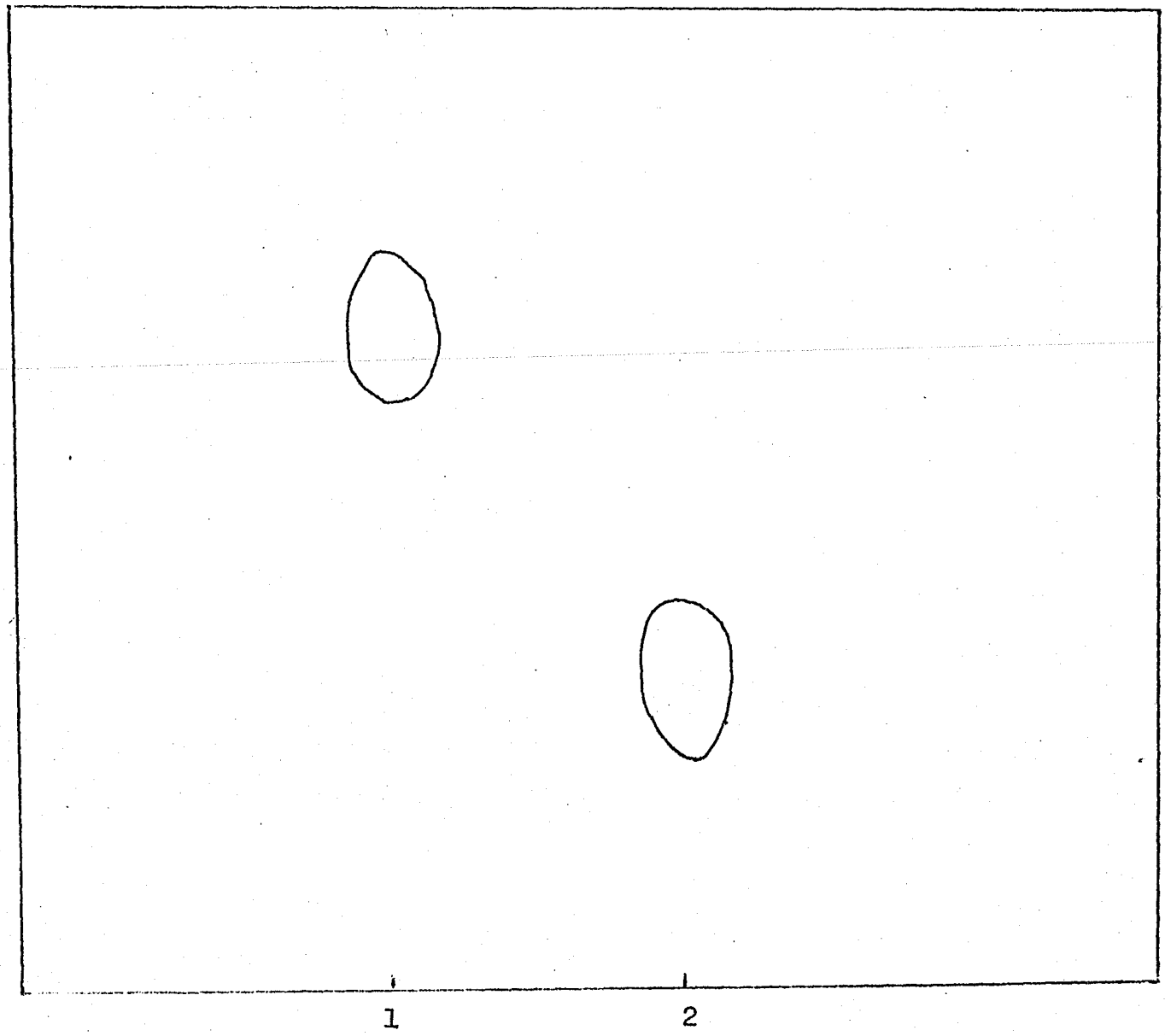


Plate No. 10

Solvent System: 100% Chloroform

Samples:
1. Benzyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside (V)
2. Cleavage products of V with KOH
3. Benzyl 4,6-O-benzylidene-α-D-glucopyranoside(II)
4. Benzyl 4,6-O-benzylidene-α-D-altropyranoside (VI)